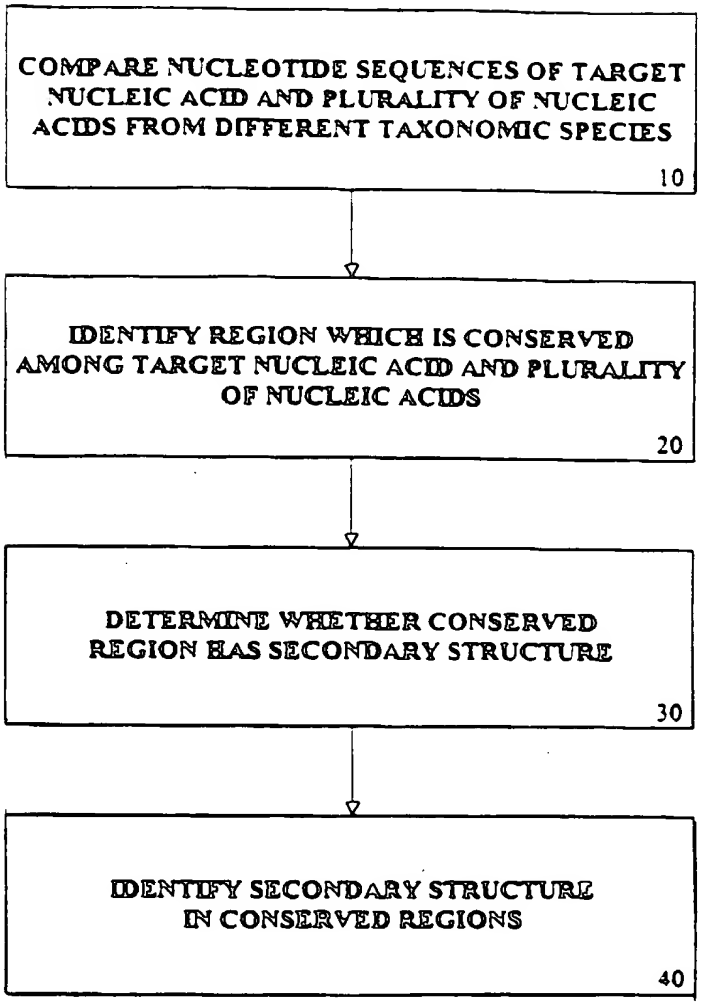

COMPARE NUCLEOTIDE SEQUENCES OF TARGET NUCLEIC ACID AND PLURALITY OF NUCLEIC ACIDS FROM DIFFERENT TAXONOMIC SPECIES
10
IDENTIFY REGION WHICH IS CONSERVED AMONG TARGET NUCLEIC ACID AND PLURALITY OF NUCLEIC ACIDS
20
DETERMINE WHETHER CONSERVED REGION HAS SECONDARY STRUCTURE
30
IDENTIFY SECONDARY STRUCTURE IN CONSERVED REGIONS
40

FIGURE 1

Find Neighbors and Assemble Flow Diagram

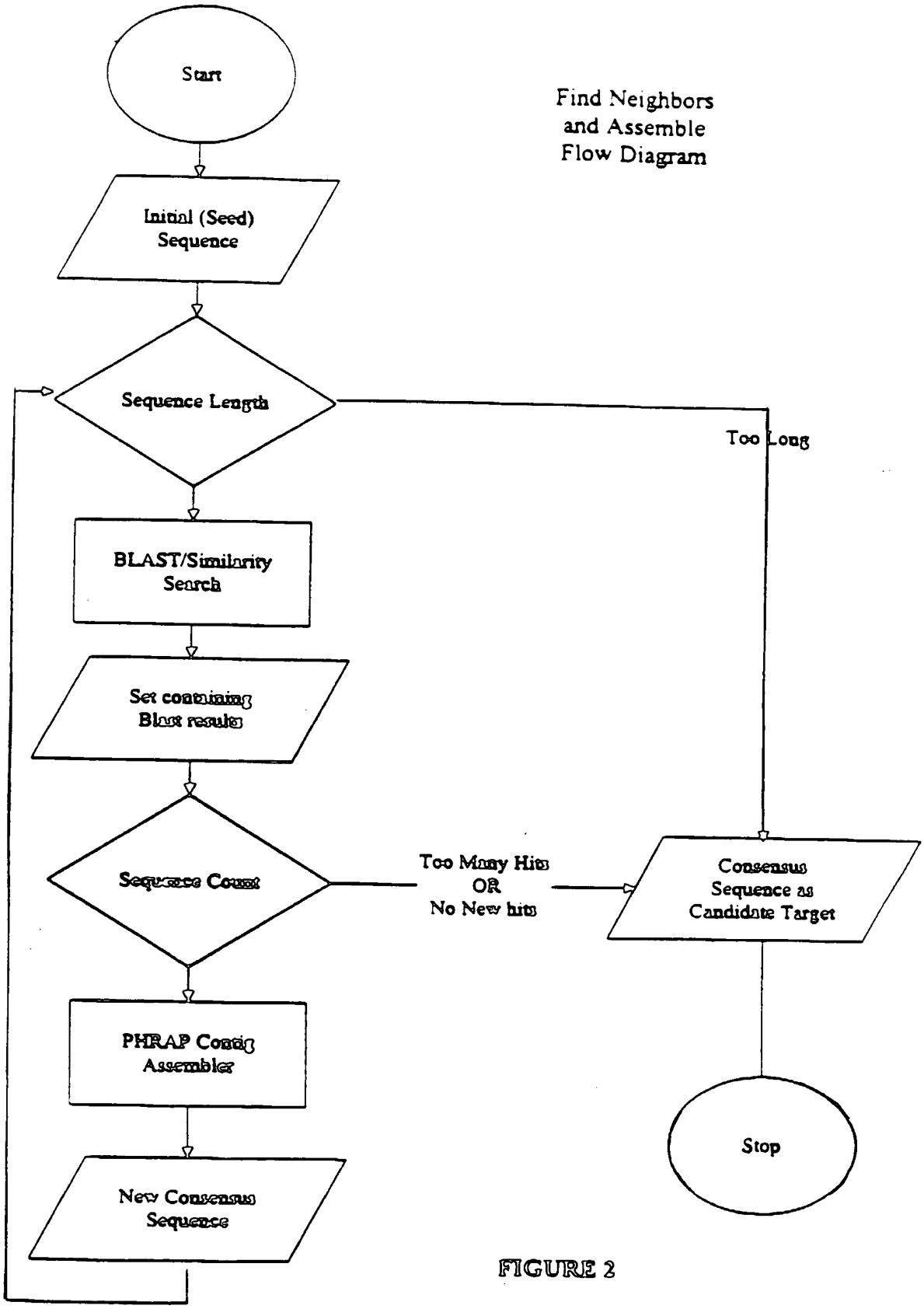


FIGURE 2

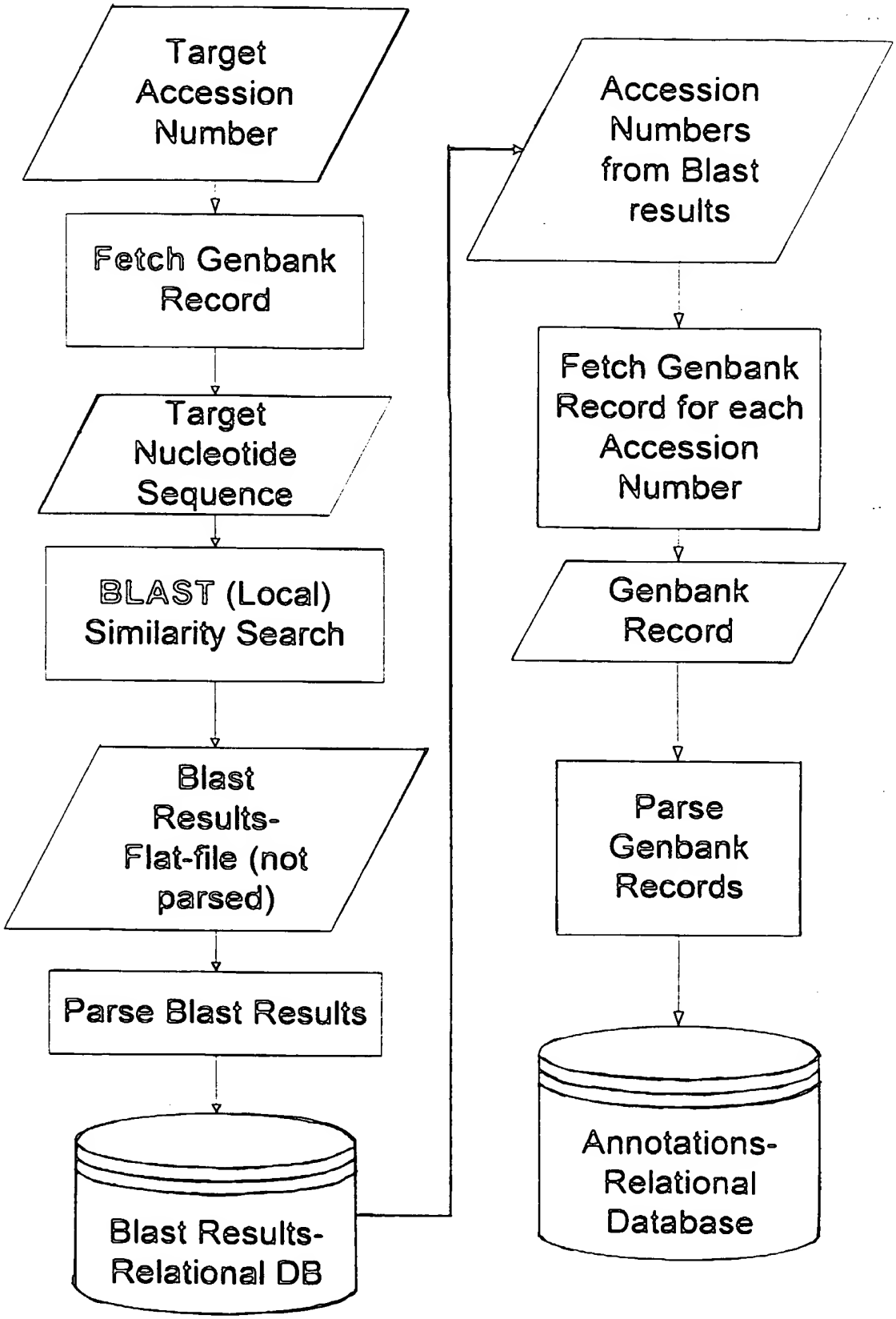

Target Accession Number
Fetch Genbank Record
Target Nucleotide Sequence
BLAST (Local) Similarity Search
Blast Results- Flat-file (not parsed)
Parse Blast Results
Blast Results- Relational DB
Accession Numbers from Blast results
Fetch Genbank Record for each Accession Number
Genbank Record
Parse Genbank Records
Annotations- Relational Database

Figure 3

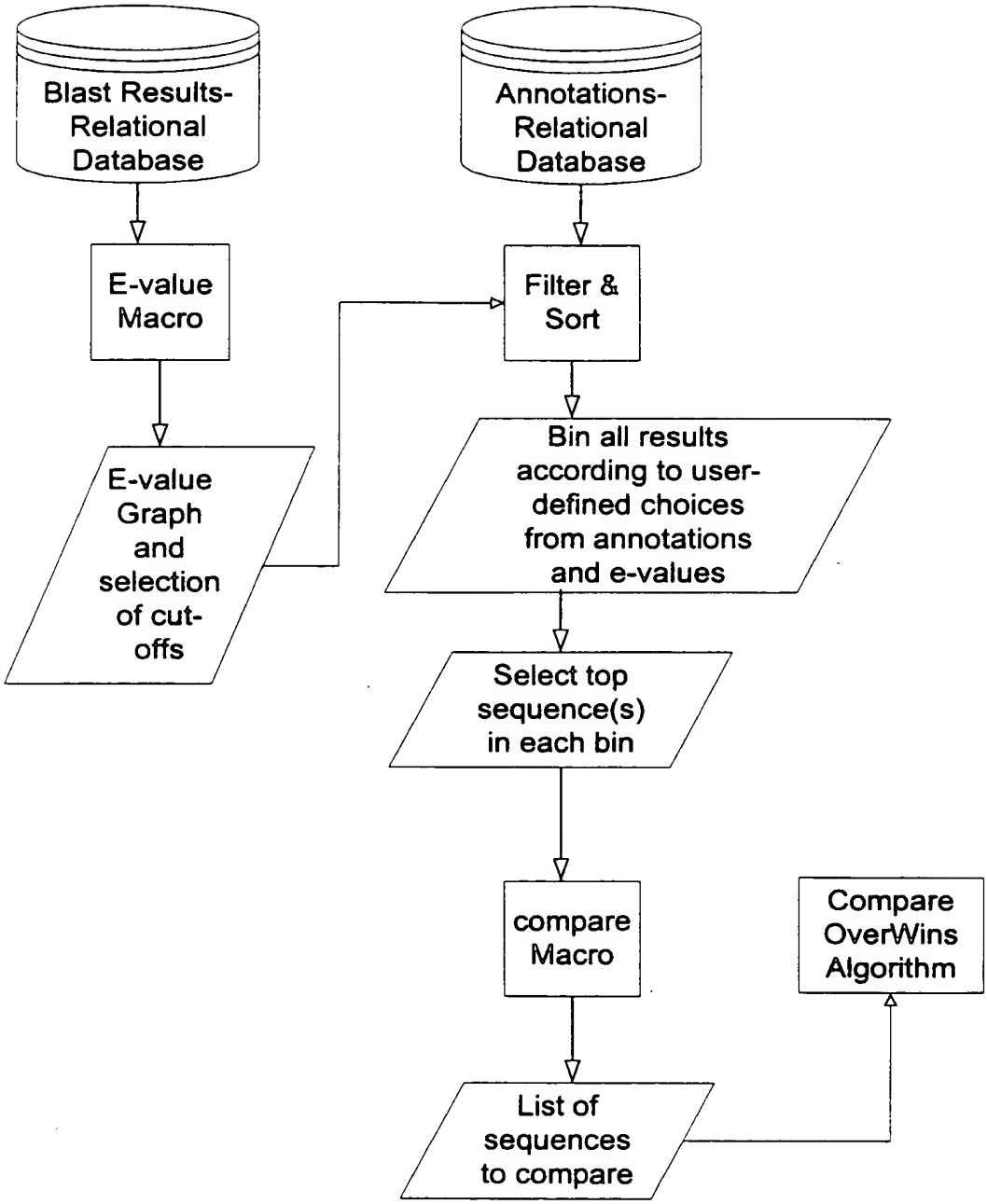

Blast Results-Relational Database
Annotations-Relational Database
E-value Macro
Filter & Sort
E-value Graph and selection of cut-offs
Bin all results according to user-defined choices from annotations and e-values
Select top sequence(s) in each bin
compare Macro
Compare OverWins Algorithm
List of sequences to compare

FIGURE 4

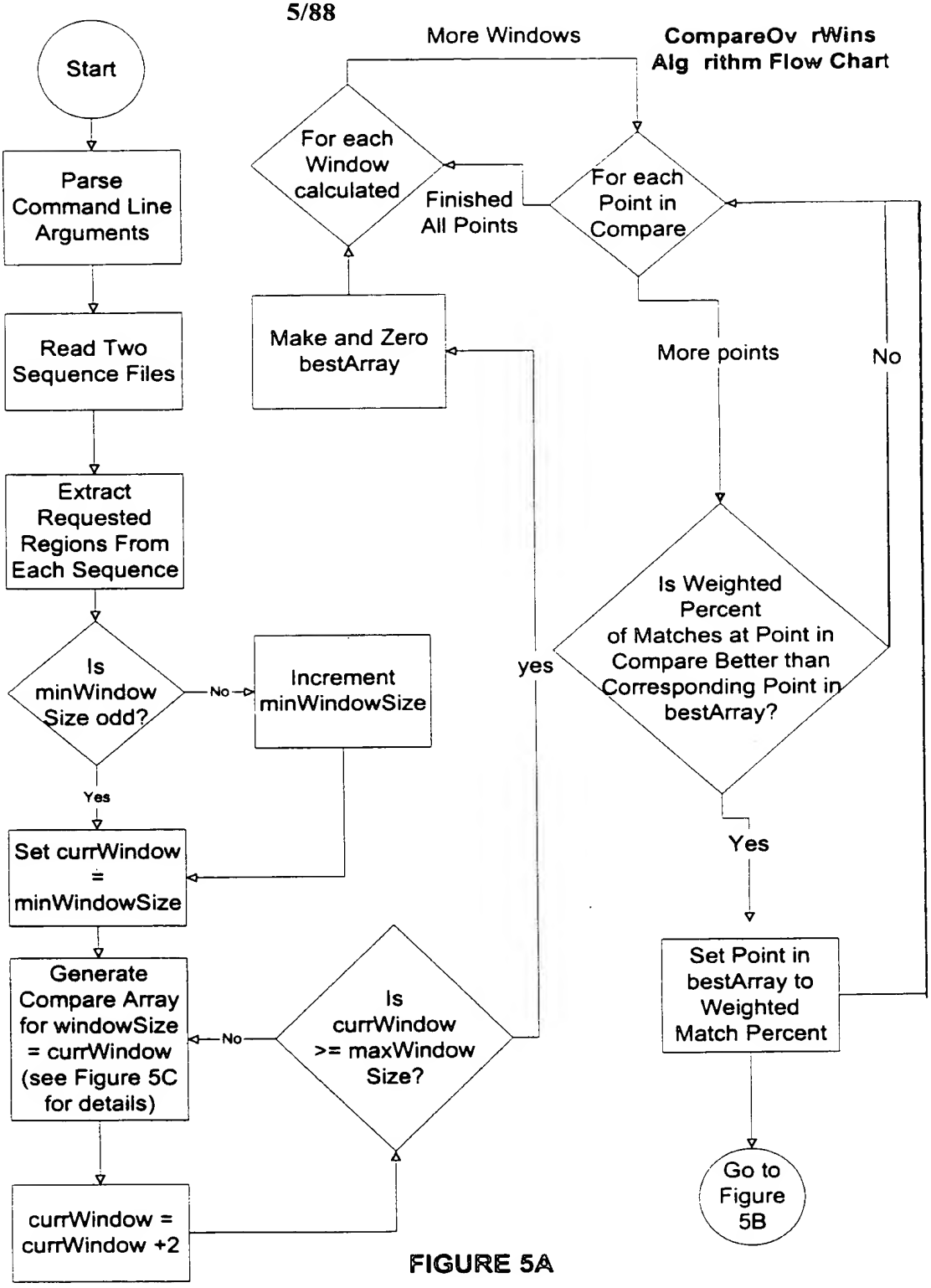

CompareOv rWins Alg rithm Flow Chart
Start
Parse Command Line Arguments
Read Two Sequence Files
Extract Requested Regions From Each Sequence
Is minWindow Size odd?
No
Increment minWindowSize
Yes
Set currWindow = minWindowSize
Generate Compare Array for windowSize = currWindow (see Figure 5C for details)
currWindow = currWindow +2
Is currWindow >= maxWindow Size?
No
yes
Make and Zero bestArray
For each Window calculated
Finished All Points
More Windows
For each Point in Compare
More points
No
Is Weighted Percent of Matches at Point in Compare Better than Corresponding Point in bestArray?
Yes
Set Point in bestArray to Weighted Match Percent
Go to Figure 5B

FIGURE 5A

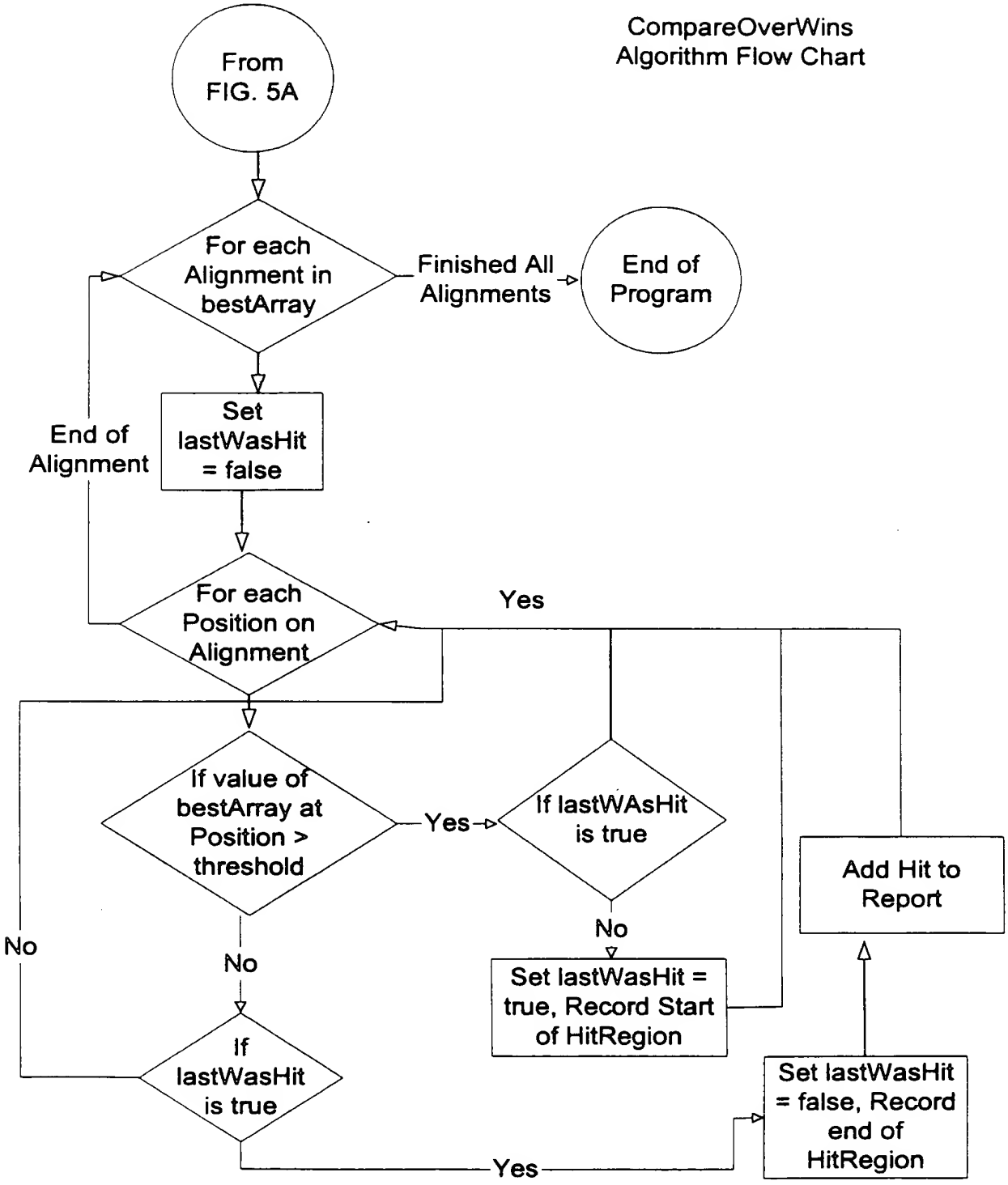

CompareOverWins
Algorithm Flow Chart
From FIG. 5A
For each Alignment in bestArray
Finished All Alignments
End of Program
End of Alignment
Set lastWasHit = false
For each Position on Alignment
Yes
If value of bestArray at Position > threshold
Yes
If lastWAsHit is true
No
Add Hit to Report
No
No
Set lastWasHit = true, Record Start of HitRegion
If lastWasHit is true
Set lastWasHit = false, Record end of HitRegion
Yes

FIGURE 5B

7/88

CompareOv rWins Algorithm Flow Chart Basic Compare

Input:
Sequence A length a
Sequence B length b
Window Size

Output:
Array of size a by b of unsigned chars (0-255)
Each point represents the number of matches in the window at that allignment and position

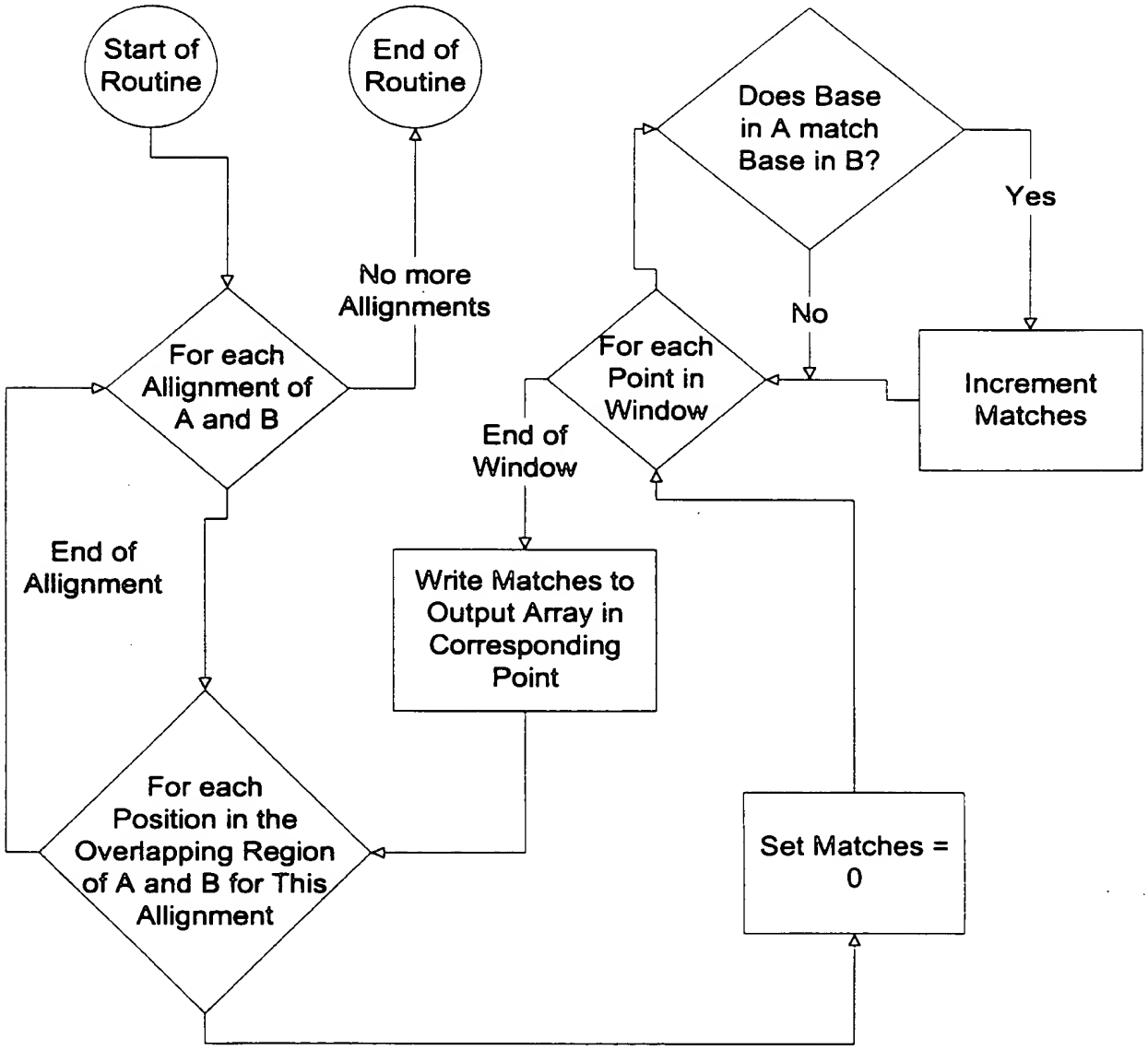
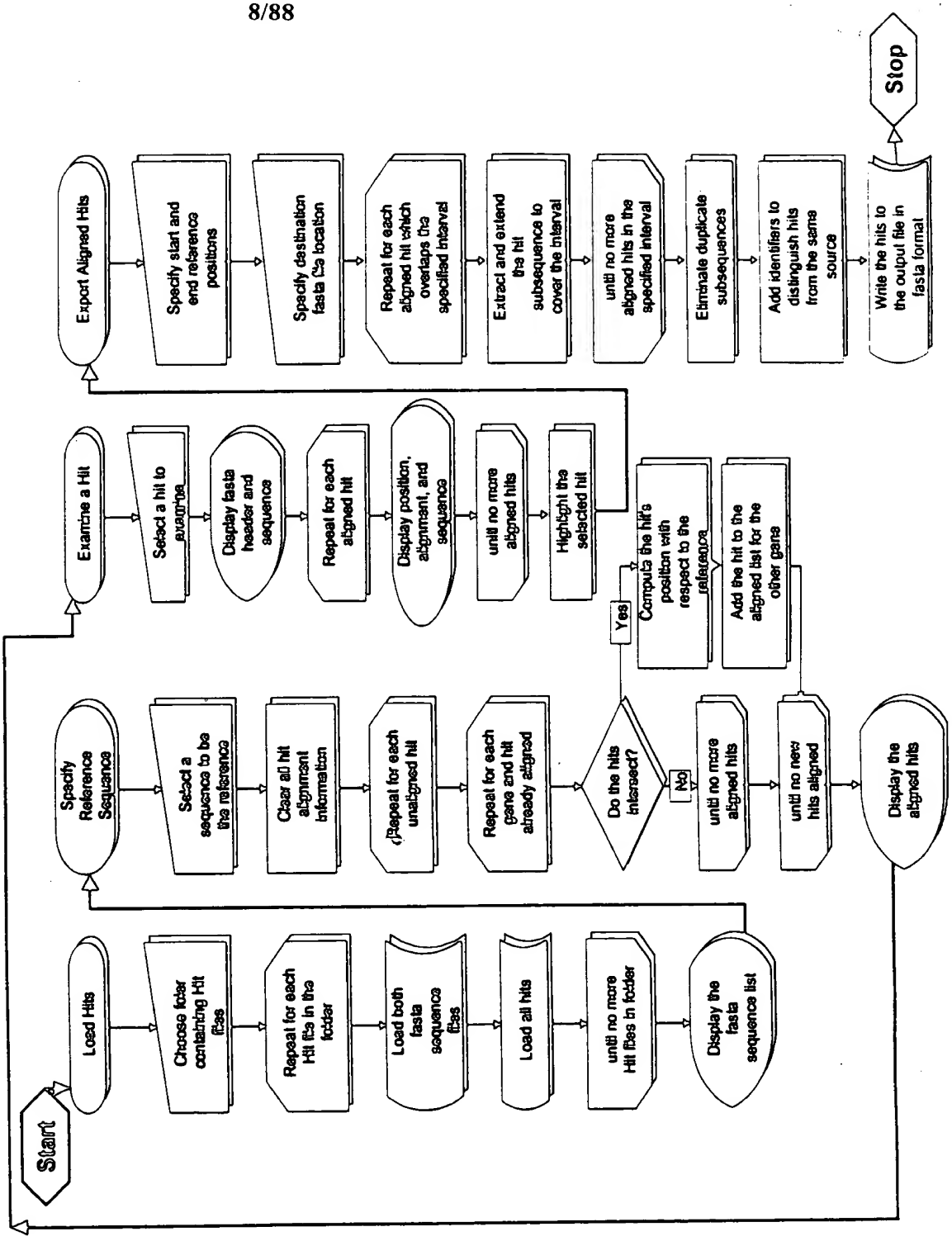


FIGURE 5C

FIGURE 5D


Start
Load Hits
Choose folder containing Hit files
Repeat for each Hit file in the folder
Load both fasta sequence files
Load all hits
until no more Hit files in folder
Display the fasta sequence list
Specify Reference Sequence
Select a sequence to be the reference
Clear all hit alignment information
Repeat for each unaligned hit
Repeat for each gene and hit already aligned
Do the hits intersect?
Yes
No
Compute the hit's position with respect to the reference
Add the hit to the aligned list for the other gene
until no more aligned hits
until no new hits aligned
Display the aligned hits
Examine a Hit
Select a hit to examine
Display fasta header and sequence
Repeat for each aligned hit
Display position, alignment, and sequence
until no more aligned hits
Highlight the selected hit
Export Aligned Hits
Specify start and end reference positions
Specify destination fasta file location
Repeat for each aligned hit which overlaps the specified interval
Extract and extend the hit subsequence to cover the interval
until no more aligned hits in the specified interval
Eliminate duplicate subsequences
Add identifiers to distinguish hits from the same source
Write the hits to the output file in fasta format
Stop

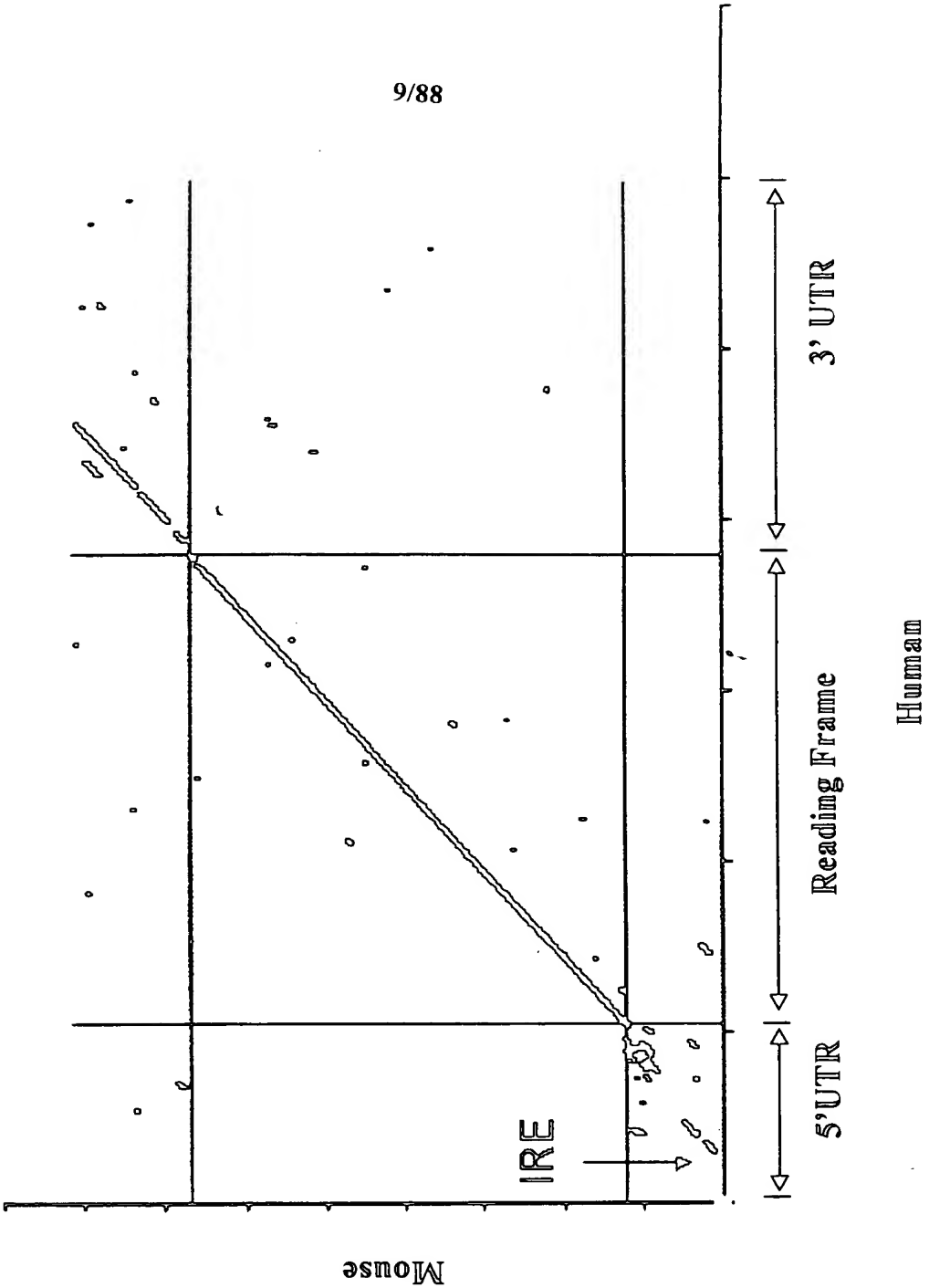


Figure 6

Self Complementation Analysis - Single Sequence

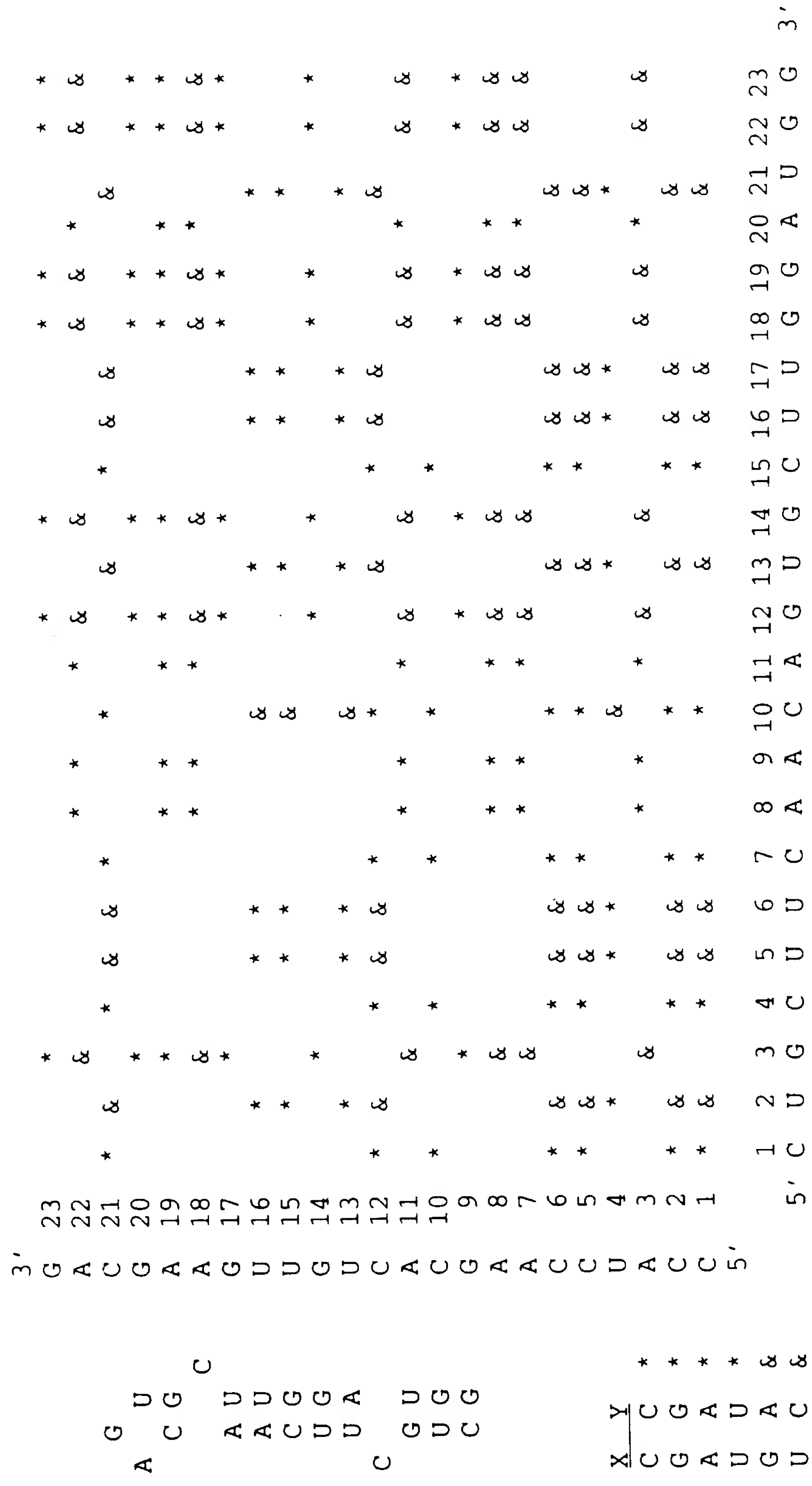


Figure 7

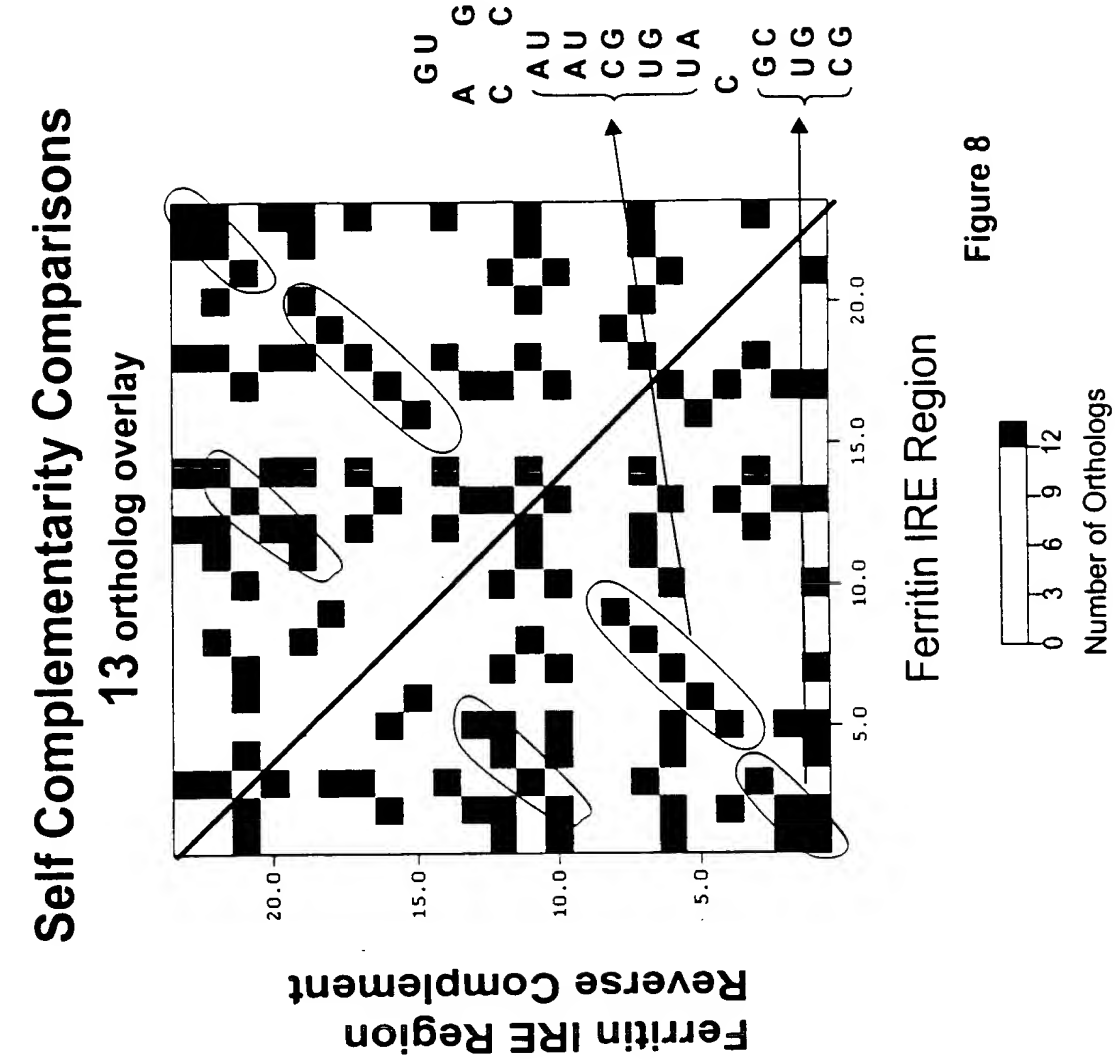

Self Complementarity Comparisons
13 ortholog overlay
Ferritin IRE Region
Reverse Complement
20.0
15.0
10.0
5.0
G U
A C
C
A U
A U
C G
U G
U A
C
G C
U G
C G
Ferritin IRE Region
20.0
15.0
10.0
5.0
0 3 6 9 12
Number of Orthologs

Figure 8

IRE String descriptor
This descriptor allows for
• a wobble (W) of 2
• no mismatches.
• N can be any nucleotide
• H refers to the single region
• S refers to the single stranded region.
H1 S1 H2 S2 H2 H1
H1 3:3 NNN:NNN
S1 1 C
H2 5:5 NNNNN:NNNNN
S2 6 CAGNGN
W2
M0
IRE
Stem-loop Model

Figure 9

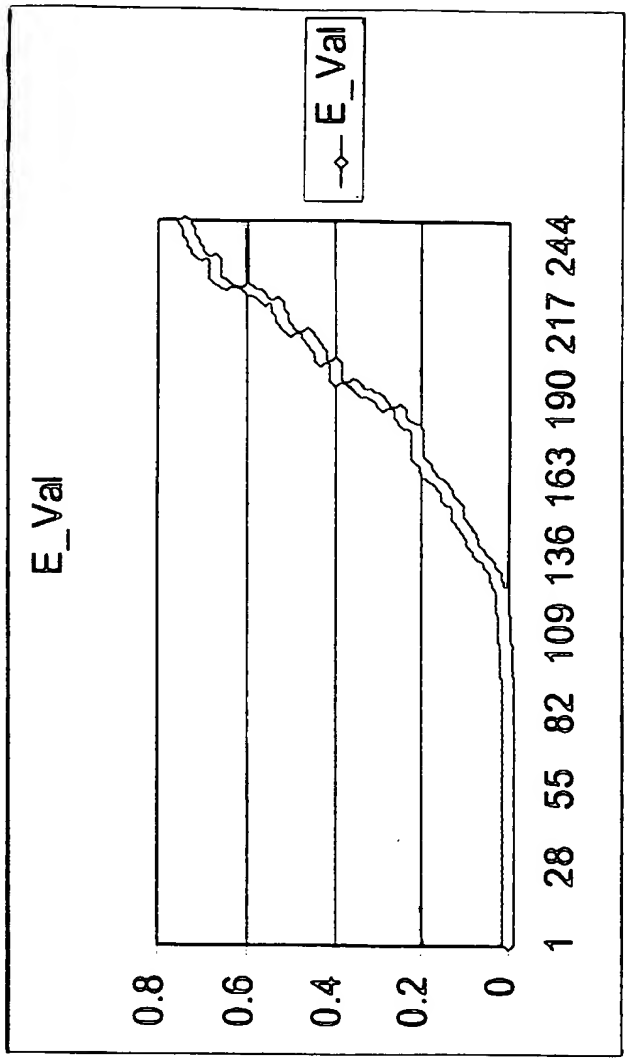


Figure 10

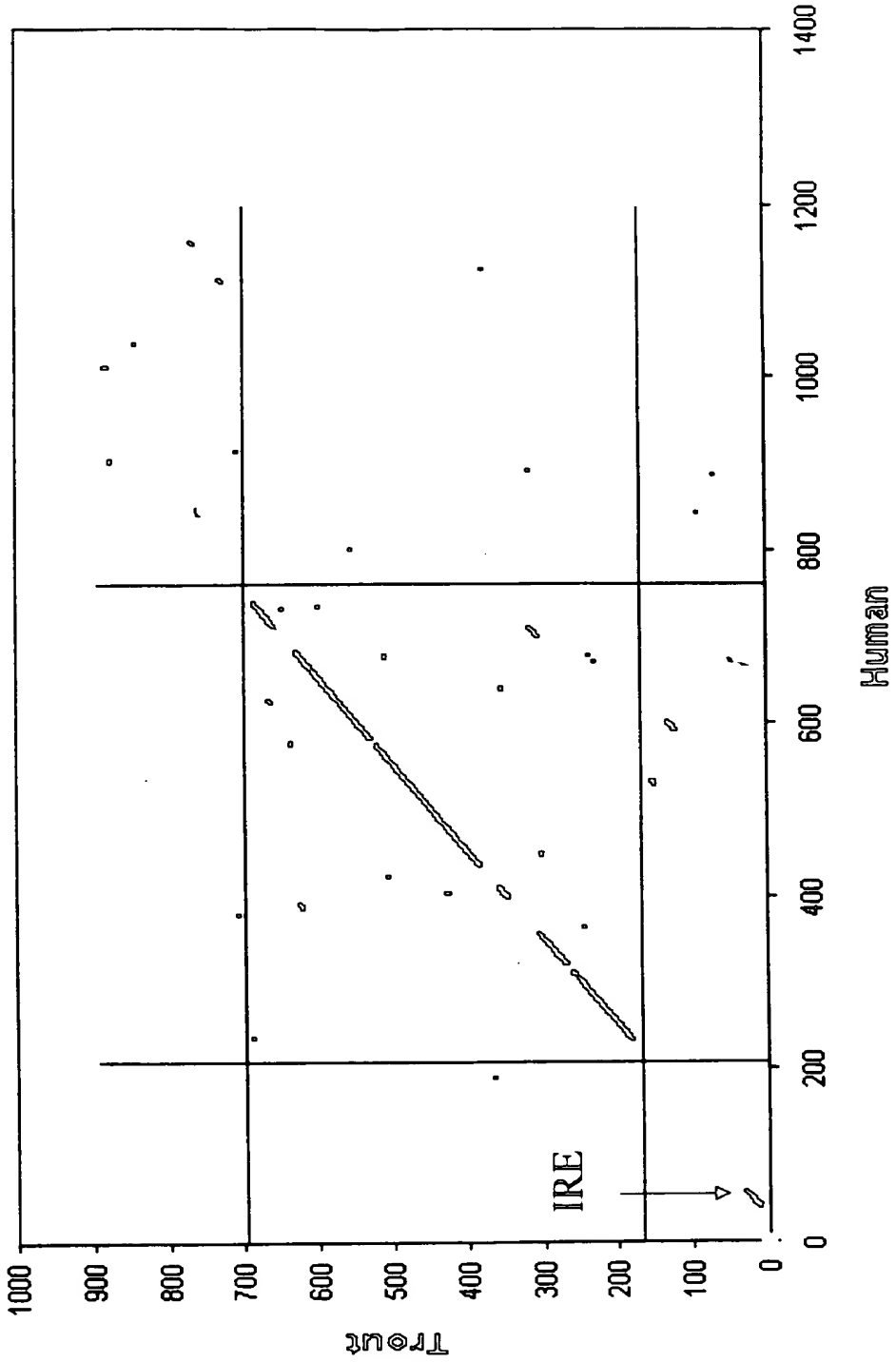

Trout
1000
900
800
700
600
500
400
300
200
100
0
IRE
0
200
400
600
800
1000
1200
1400
Human

Figure 11

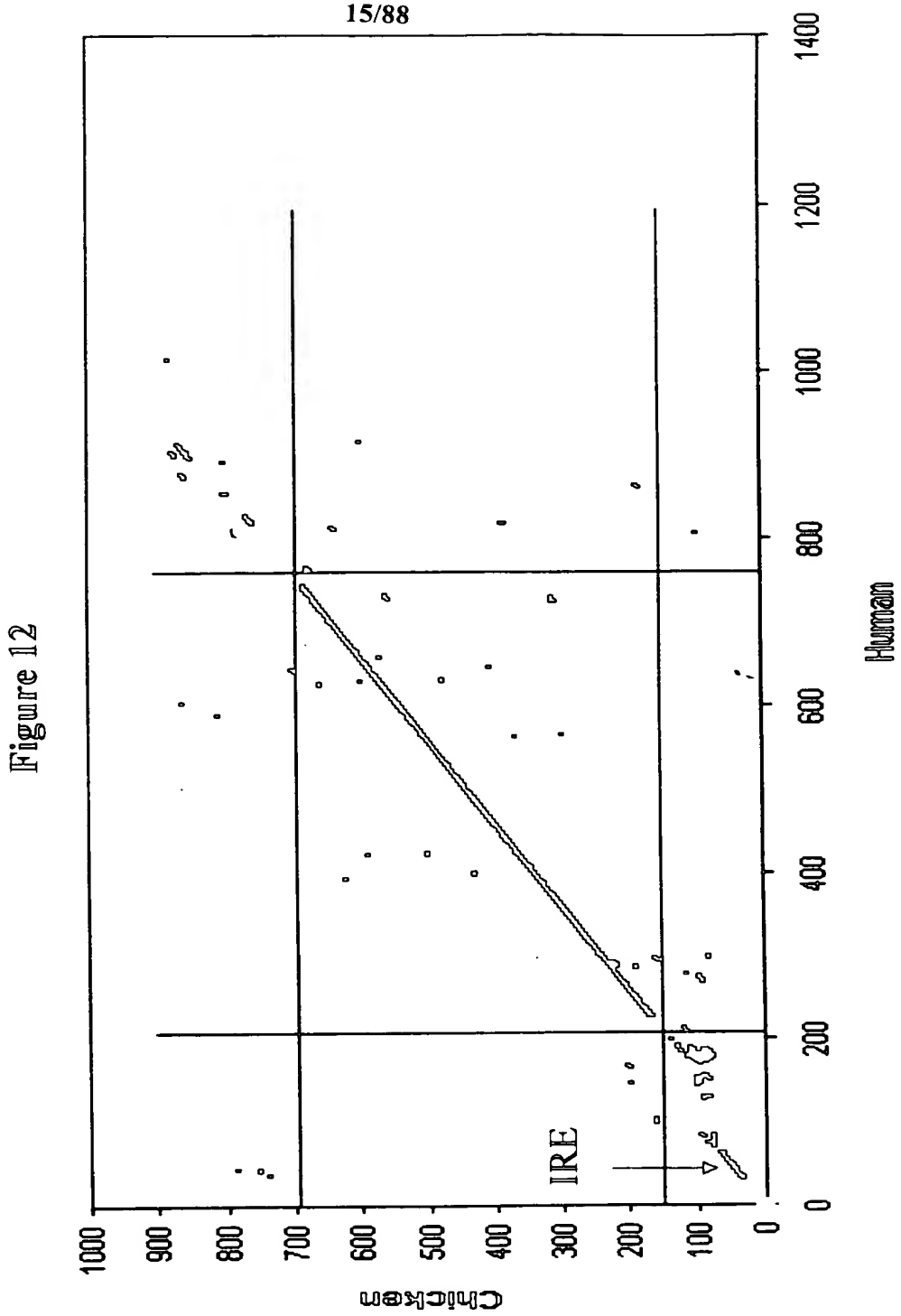
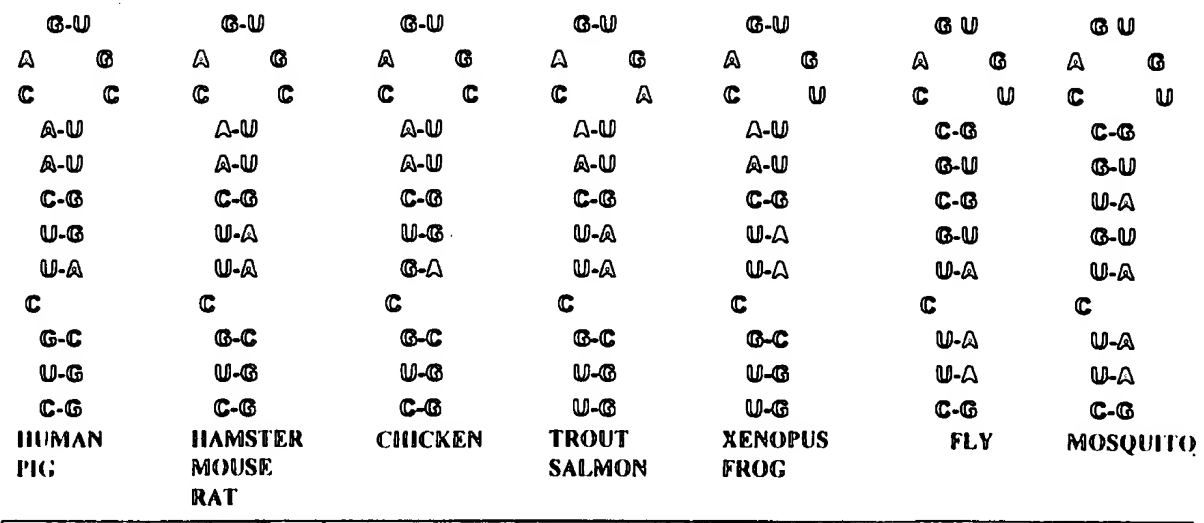

Chicken
Human
IRE
0
100
200
300
400
500
600
700
800
900
1000
0
200
400
600
800
1000
1200
1400

Figure 12

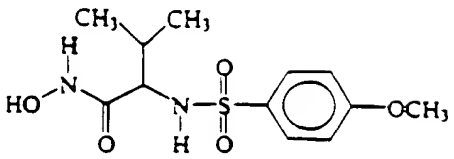


	HUMAN PIG	HAMSTER MOUSE RAT	CHICKEN	TROUT SALMON	XENOPUS FROG	FLY	MOSQUITO
HUMAN PIG	No	No	Yes	Yes	Yes	No	No
HAMSTER MOUSE RAT		No	Yes	Yes	Yes	No	No
CHICKEN			No	Yes	Yes	No	No
TROUT SALMON				No	Yes	Yes	Yes
XENOPUS FROG					No	Yes	Yes
FLY						No	Yes
MOSQUITO							No

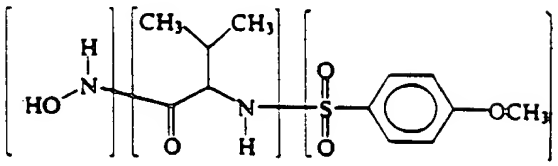
Figure 13

17/88

Compound CI



$C_{12}H_{18}N_2O_5S$



	F_i	F_{ii}	F_{iii}
Molecular formula	H_2NO	C_5H_9NO	$C_7H_7O_3S$

Figure 14

Addition of fragments to yield compounds

Table

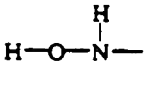
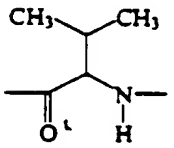
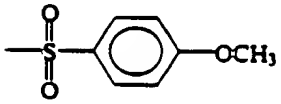
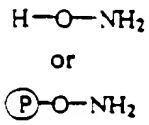
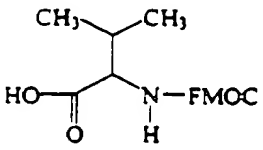
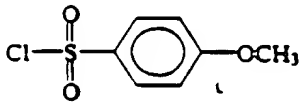
Fragment Identifier	Structure	Name	Molecular formula	Other
F_i		Hydroxylamine	H_2NO	...
F_{ii}		Amino acid	C_5H_9NO	...
F_{iii}		Sulfonyl	$C_7H_7O_3S$	...

Figure 15

Reagents	Identifier	Name	Properties
	R_i	Hydroxylamine	...
	R_{ii}	FMOC blocked amino acid	...
	R_{iii}	Sulfonylchloride	...

Ⓟ = Solid support

Figure 16

20/88

Transformation

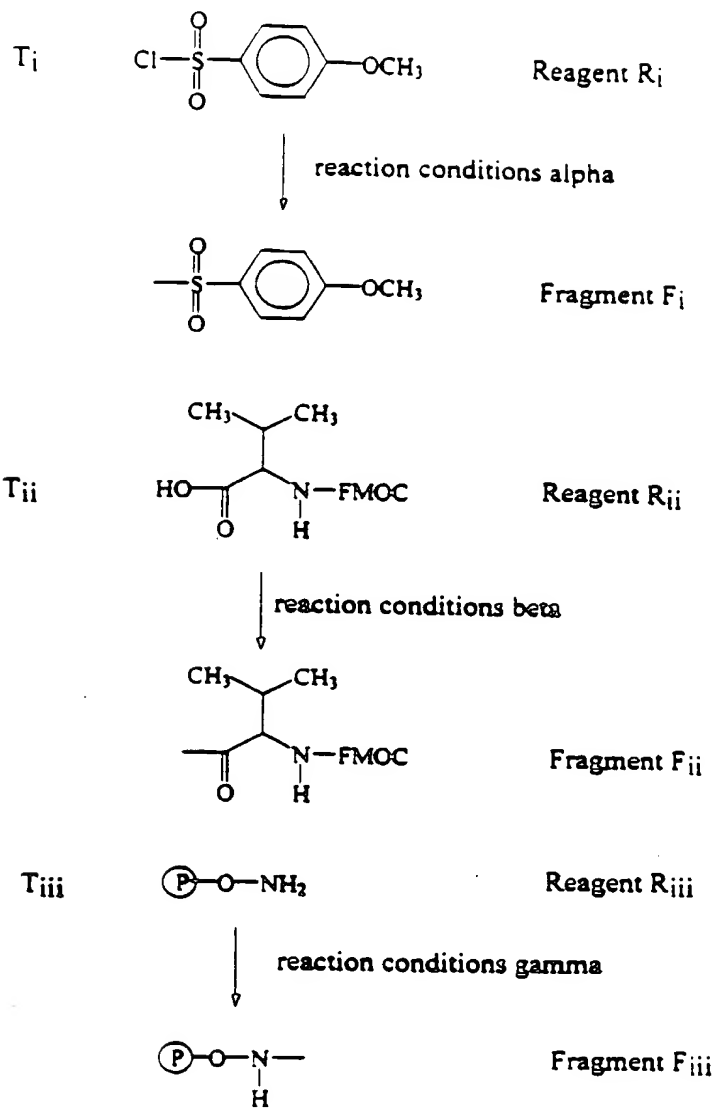


Figure 17

Common Fragment / Different Reagents and Transformations

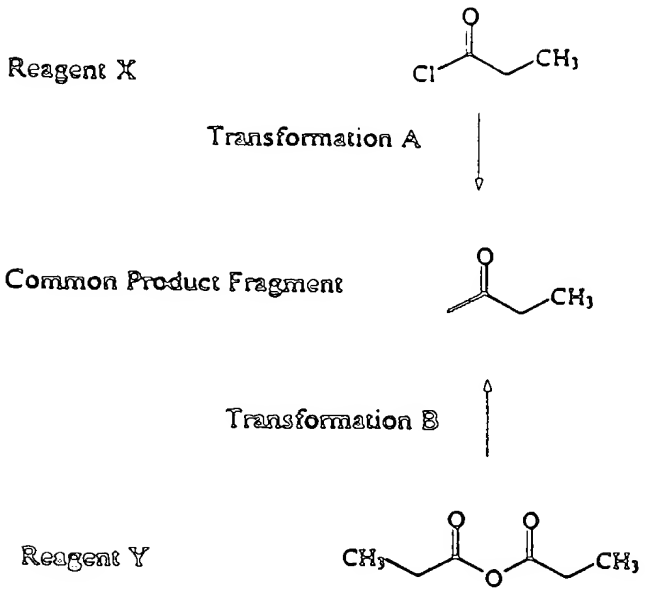


Figure 18

22/88

Common Fragment / Different Reagents and Transformations

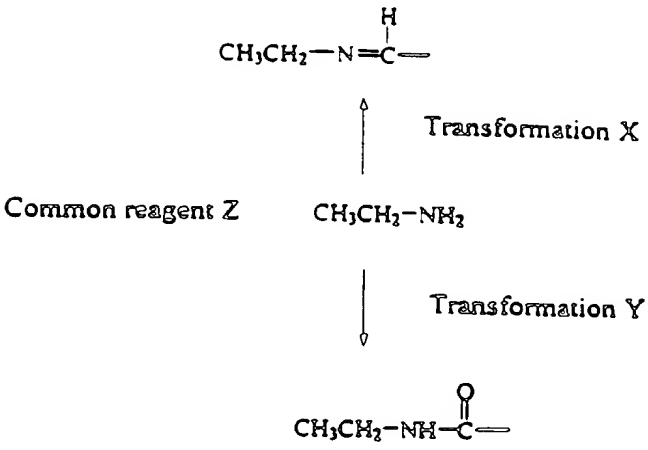


Figure 19A

23/88

Common Reagent

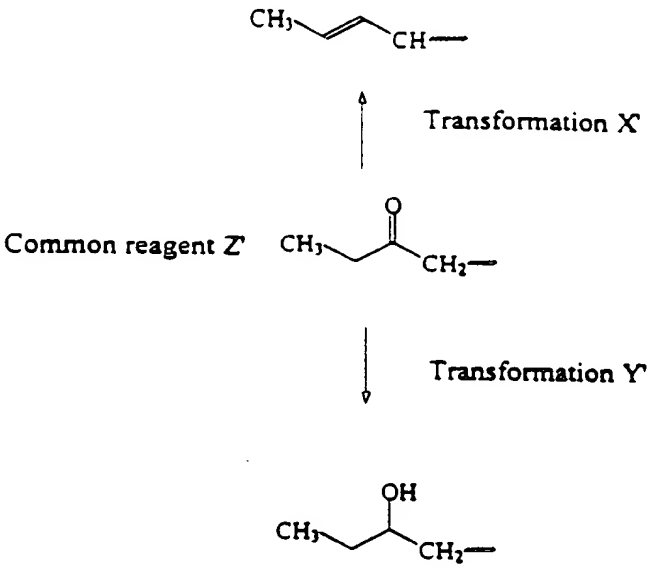
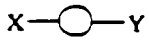
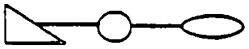


Figure 19B

Symbolic addition of fragments to yield compound

Symbolic Structure	Symbolic Identifier	Molecular formula
Fragment		
	$F_{i'}$	$C_uH_vN_w$ ...
	$F_{ii'}$	$C_uH_vN_w$ ...
	$F_{iii'}$	$C_uH_vN_w$ ...
Compound		
	**CI'**	$C_uH_vN_w$ ...

Molecular formula $F_{i'}$
+
Molecular formula $F_{ii'}$
+
Molecular formula $F_{iii'}$
= Molecular formula CI'

Figure 20

Symbolic Reagent Table

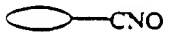
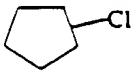
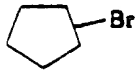
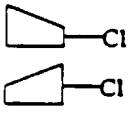
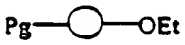
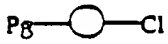
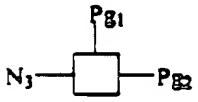
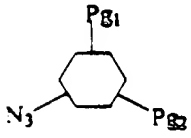
Identifier	Name	Structure	Molecular formula
R1	xxx		xxx
R2	...		...
R3	...		...
R4	...		...
R5	...		...
R6	...		...
R7	...		...
R8	...		...
R9	...		...
R10	...		...

Figure 21

Symbolic Fragment Table

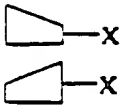
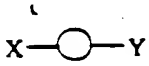
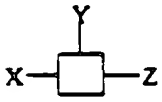
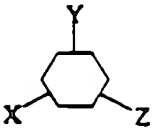
Identifier	Symbolic Structure	Molecular formula	Molecular Weight
F1		xxx	xxx
F2		...	...
F3		...	...
F4		...	...
F5		...	...
F6		...	...
F7		...	...
F8		...	...

Figure 22

27/88

Symbolic Transformation Table

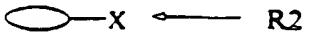
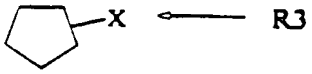
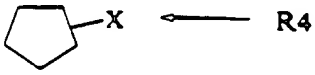
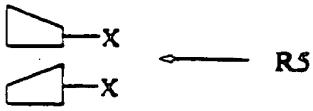
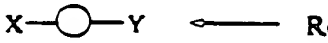
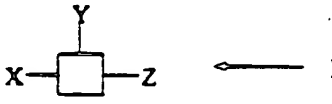
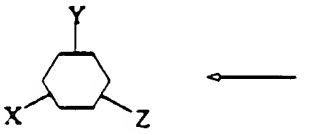
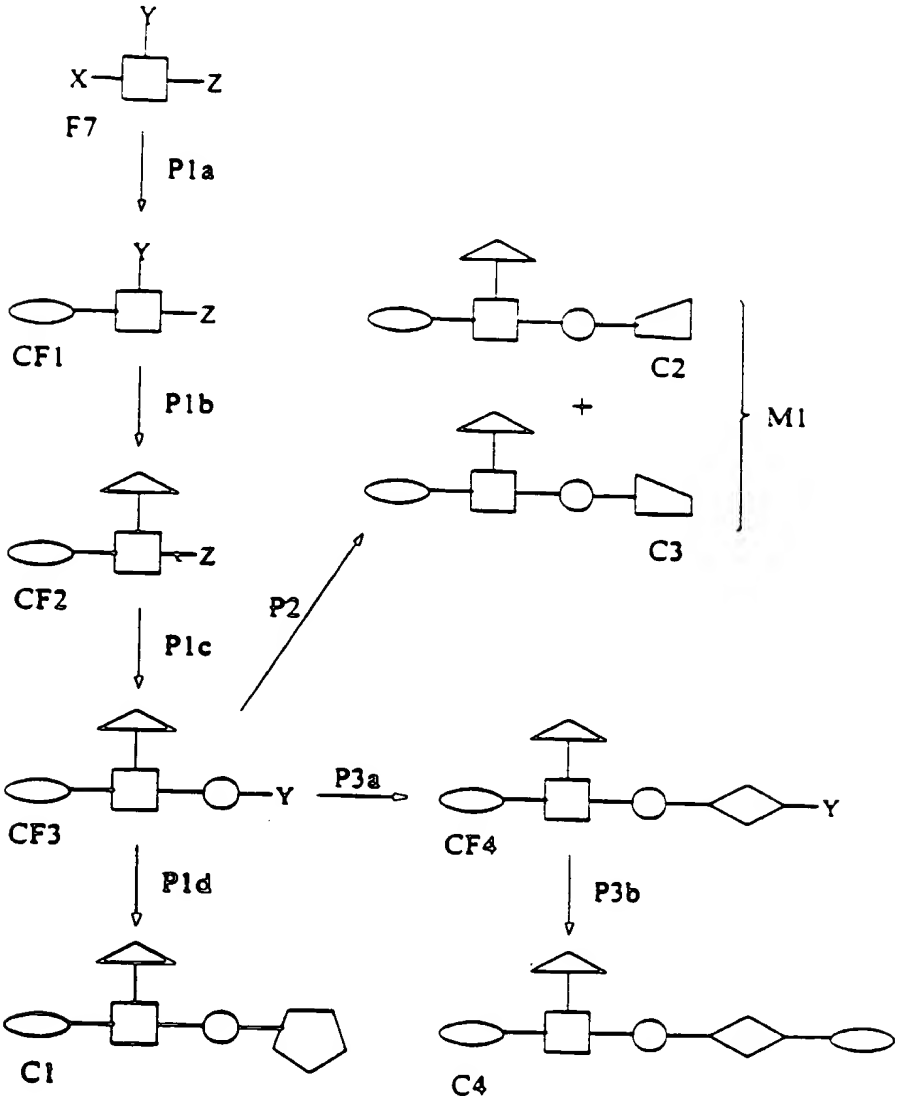
Identifier		Symbolic Reactions	Reagent
T1	F1		conditions α
T2	F2		conditions β
T3	F3		conditions α
T4	F3		conditions α
T5	F4		conditions α
T6	F5		conditions ε
T7	F5		conditions α
T8	F6		conditions α
T9	F7		conditions γ
T10	F8		conditions γ

Figure 23

28/88

Single Compounds and Mixtures



P = synthetic path
F = fragment
C = compound
CF = complex fragment
M = mixture

Figure 24

29/88

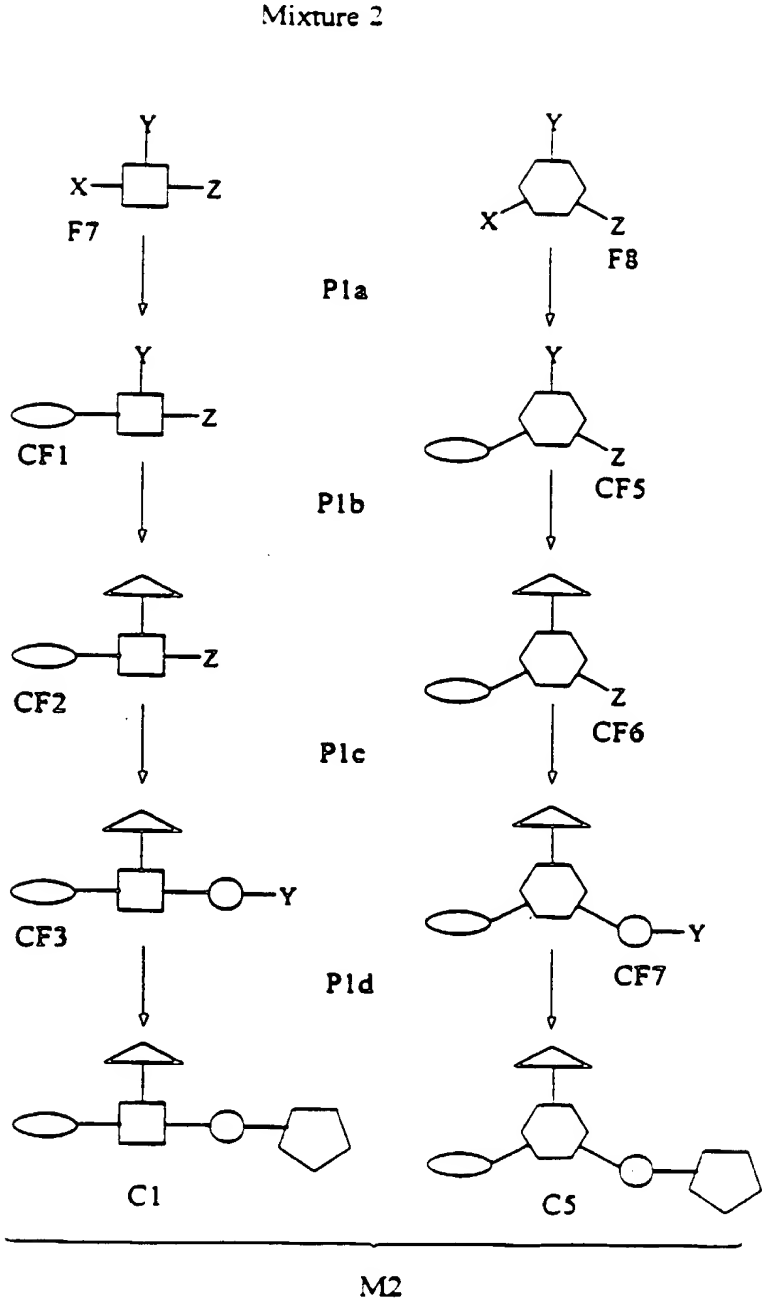


Figure 25

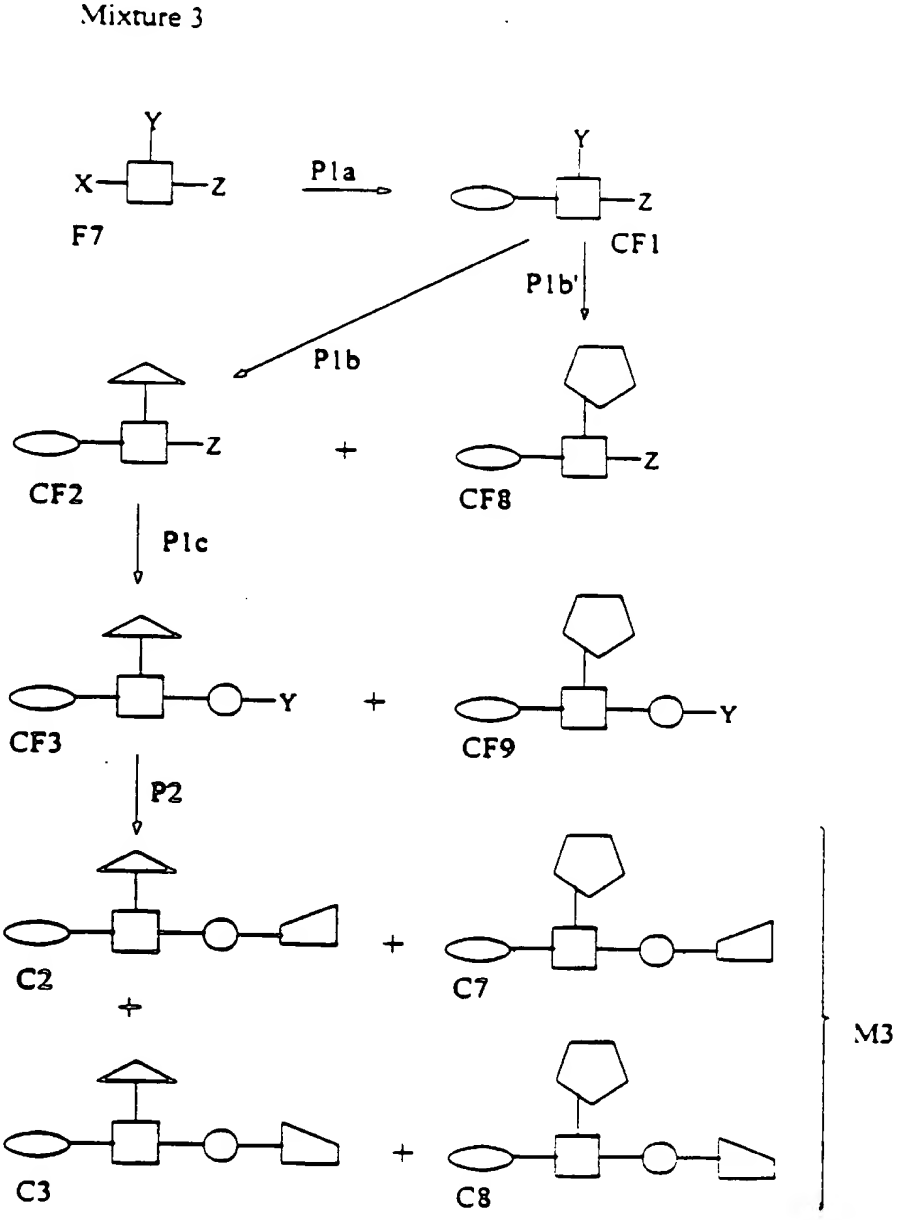
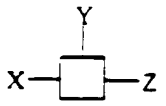

Mixture 3
Y
X
Z
F7
P1a
Y
Z
CF1
P1b'
P1b
Z
CF2
+
Z
CF8
P1c
Y
CF3
+
Y
CF9
P2
C2
+
C7
+
C3
+
C8
M3

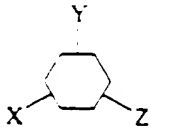
Figure 26

Mixture 4

2 Starting Fragments

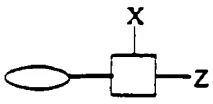


F7

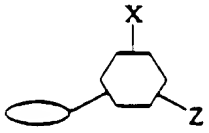


F8

2 Complex Fragments

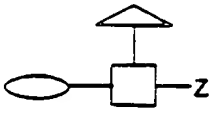


CF1

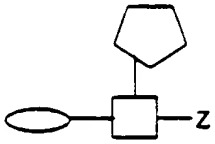


CF5

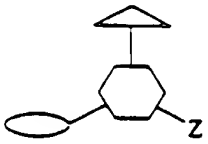
4 Complex Fragments



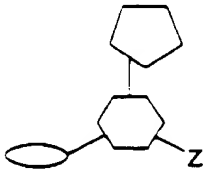
CF2



CF8

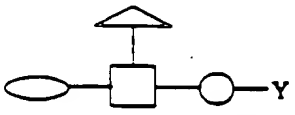


CF 6

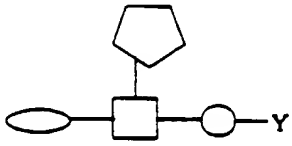


CF11

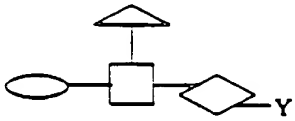
8 Complex Fragments



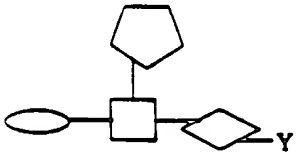
CF3



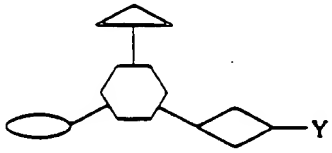
CF9



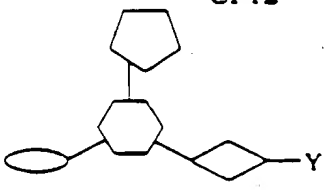
CF12



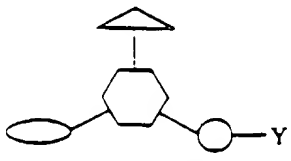
CF13



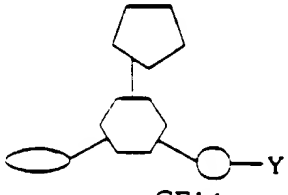
CF15



CF16



CF 7



CF14

Figure 27A

Mixture 4 (continued)

16 compounds

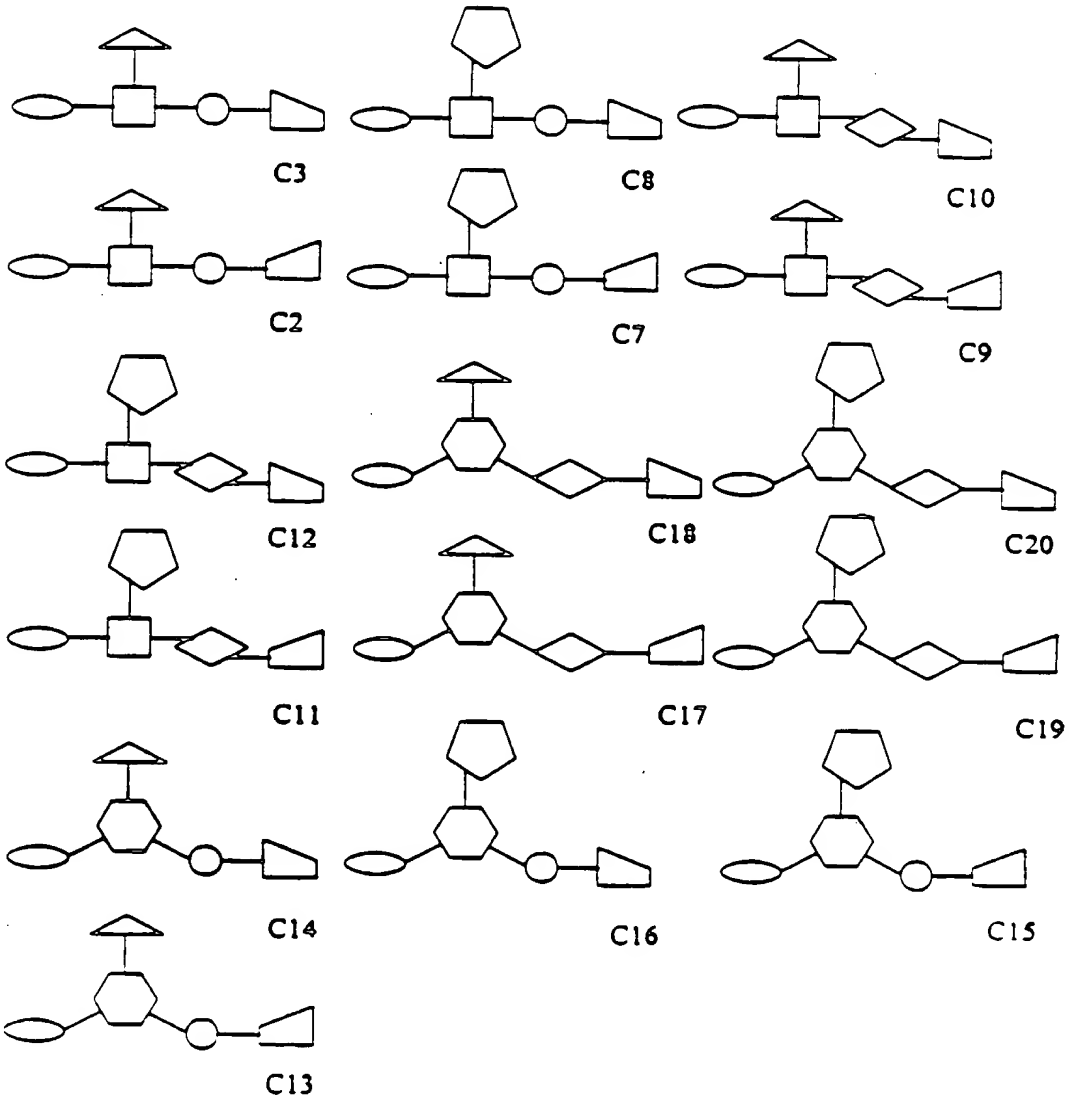
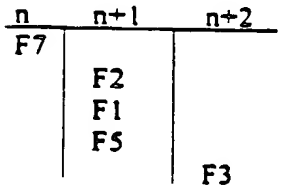


Figure 27B

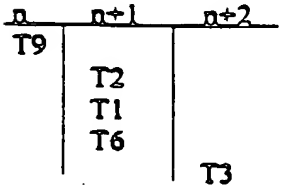
Tracking Table for Compound C1

(a) By Fragments:

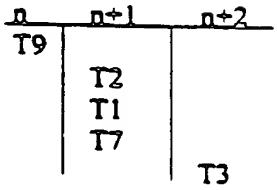


(b) By Transformations:

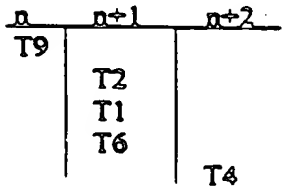
Synthesis Path 1



Synthesis Path 2



Synthesis Path 3



Synthesis Path 4

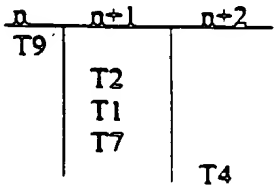


Figure 28

Tracking Table

Tracking M1

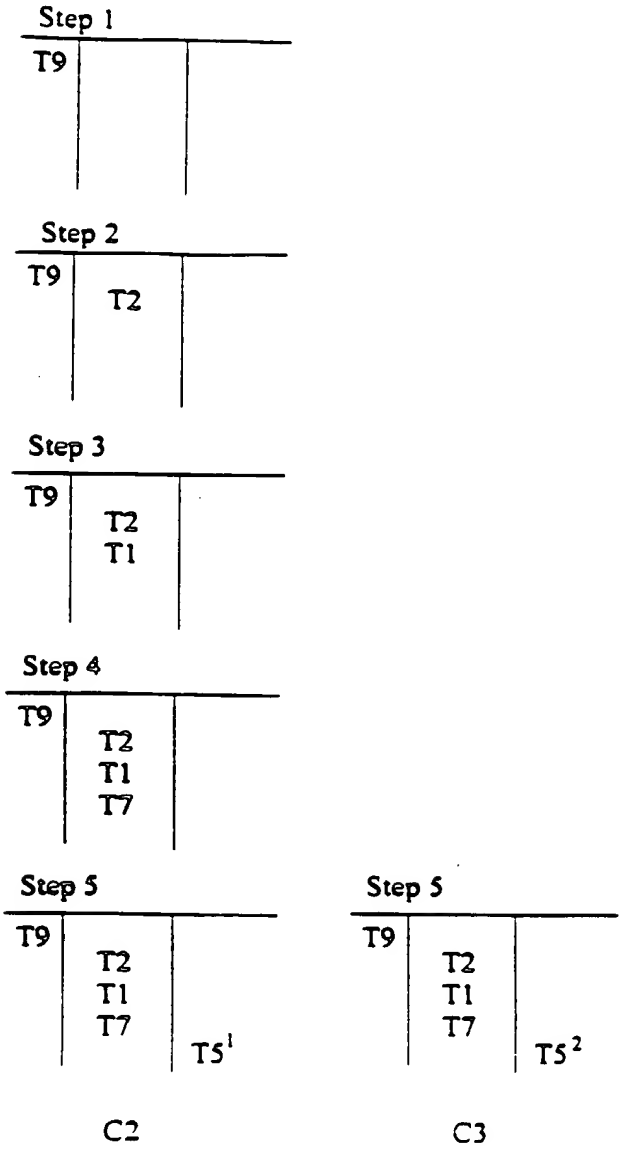
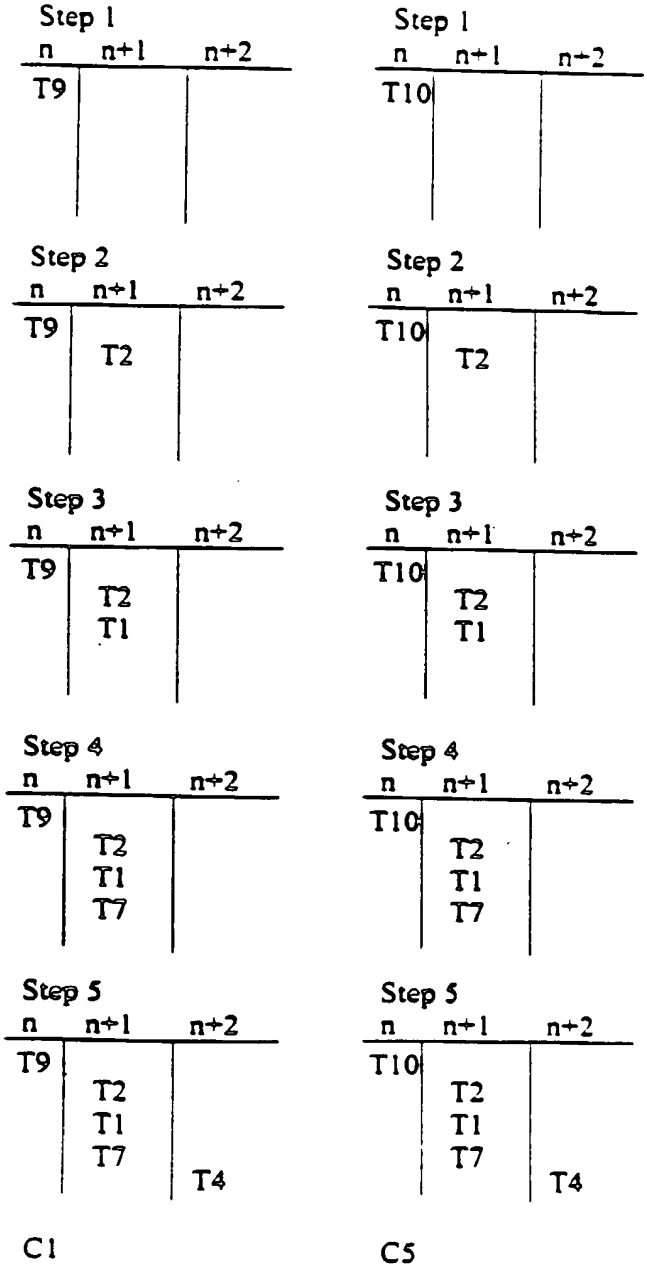


Figure 29

Tracking Table

Tracking M2



C1 C5

Figure 30

Tracking Table

Tracking M3

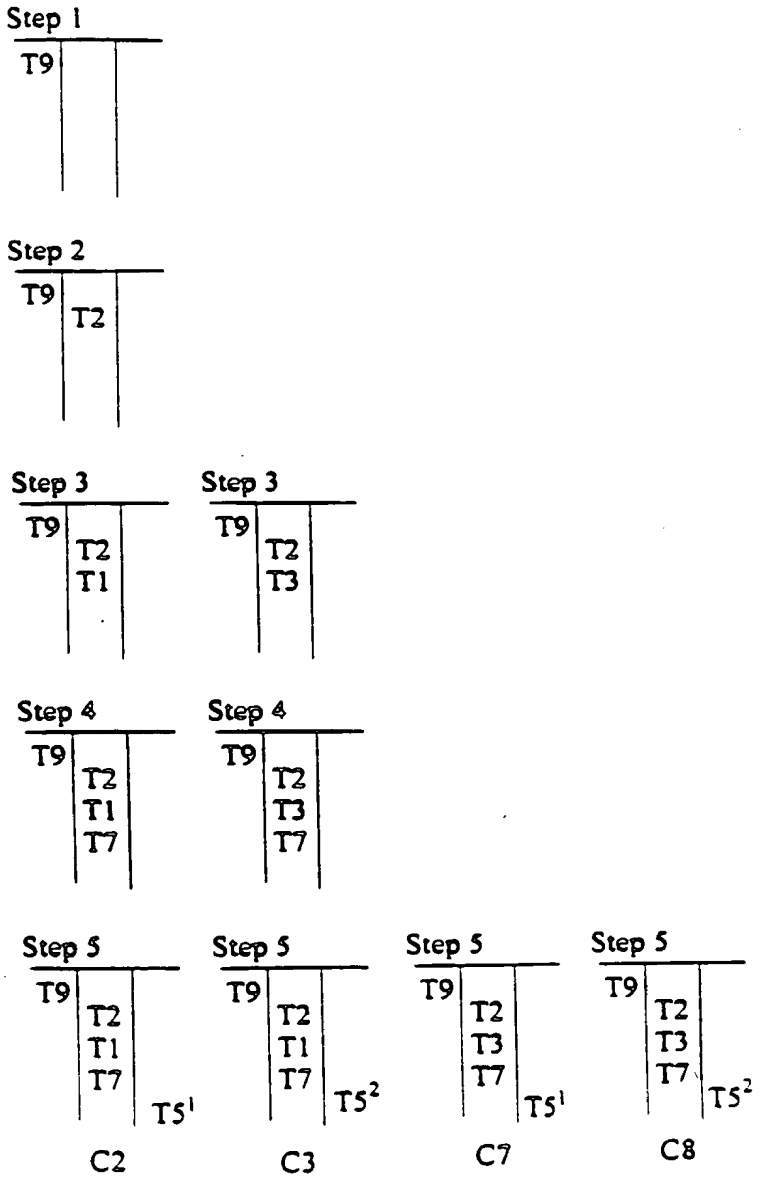


Figure 31

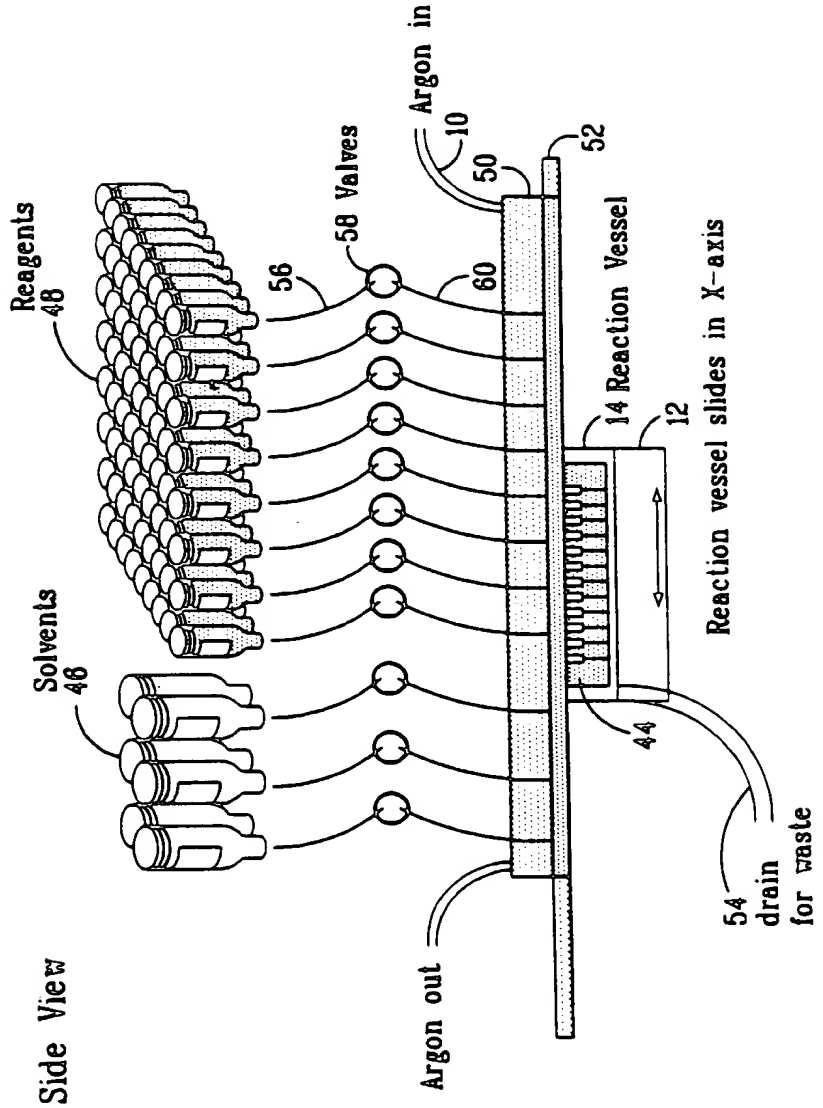

Side View
Reagents
48
Solvents
46
56
58 Valves
Argon in
10
50
52
60
14 Reaction Vessel
12
Reaction vessel slides in X-axis
44
54
drain
for waste
Argon out

Figure 32

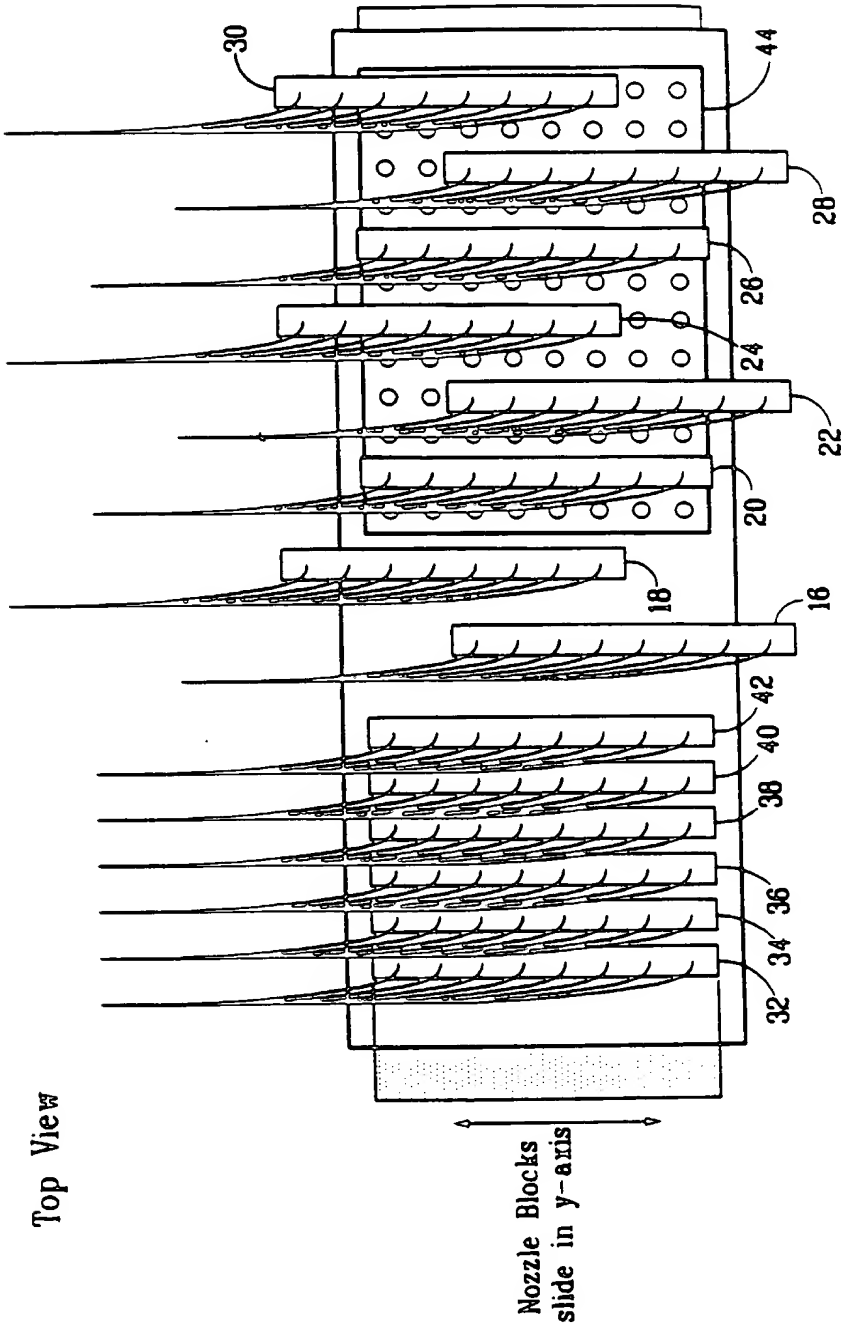

30
44
28
26
24
22
20
18
16
42
40
38
36
34
32
Top View
Nozzle Blocks slide in y-axis
Plate assembly slides in X-axis

Figure 33

39/88

Synthesis of hydroxamic acids from alcohol resin

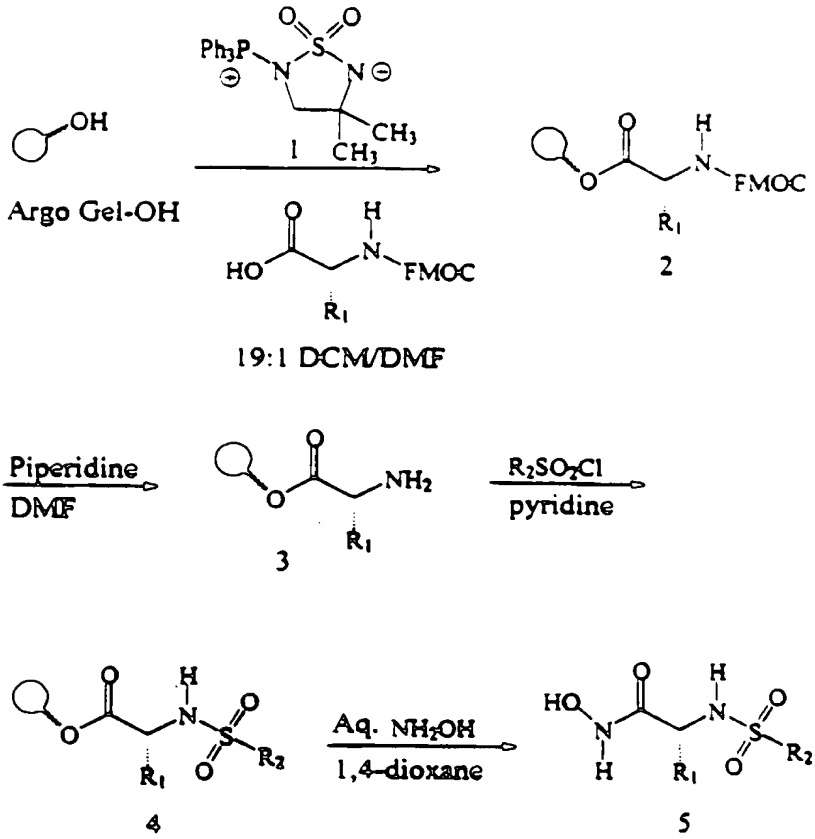
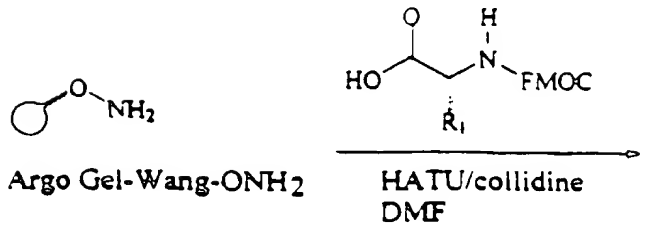
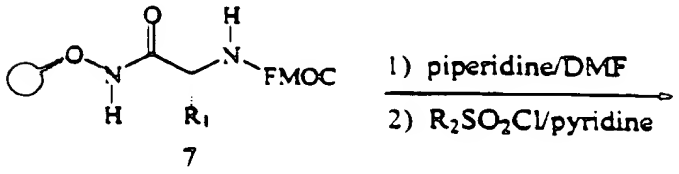


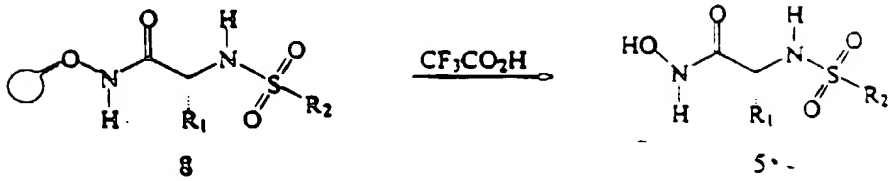
Figure 34

Synthesis of hydroxamic acids from hydroxylamine resin





7



8

5

Figure 35

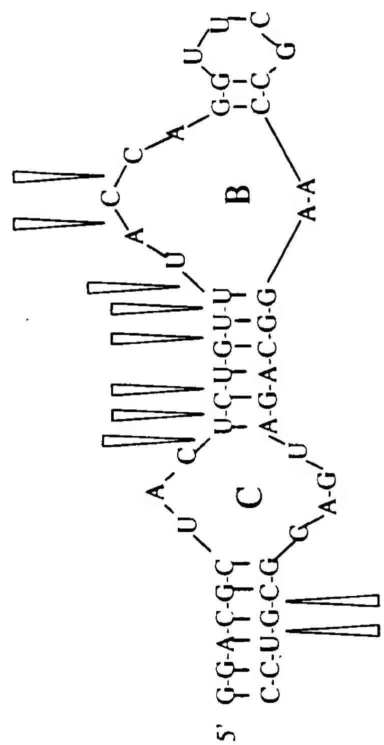


Figure 36

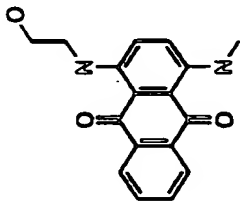
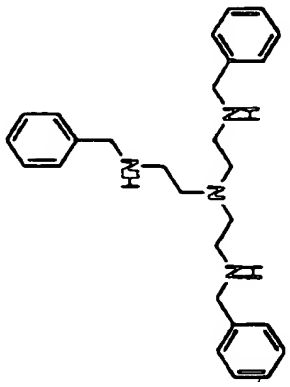
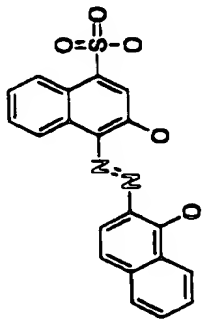
ACD Code	Structure	Calc. ΔG of binding (kcal/mole)	IC_{50} (μM)
00001199		-5.1	< 2
00192509		-8.5	< 2
00003934		-5.1	< 50

Figure 37

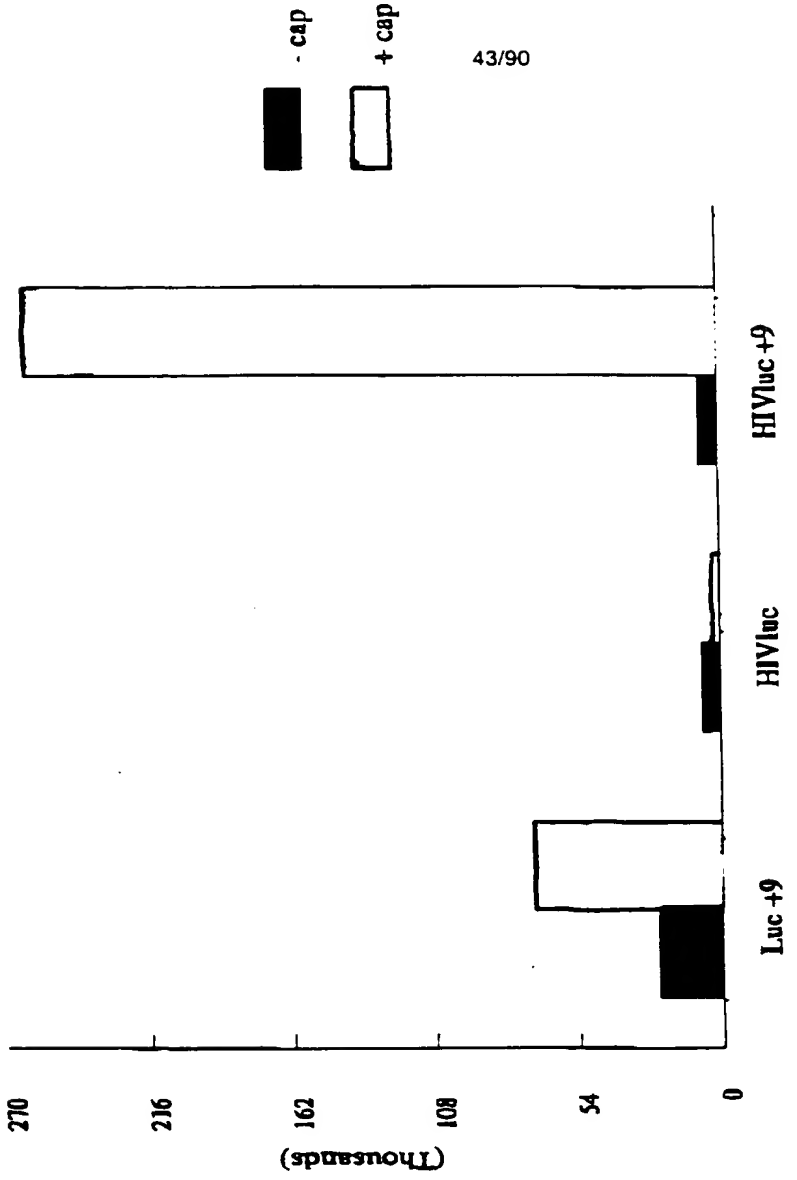

- cap
+ cap
43/90
HIVluc+9
HIVluc
Luc+9
270
216
162
108
54
0
(Thousands)

Figure 38A

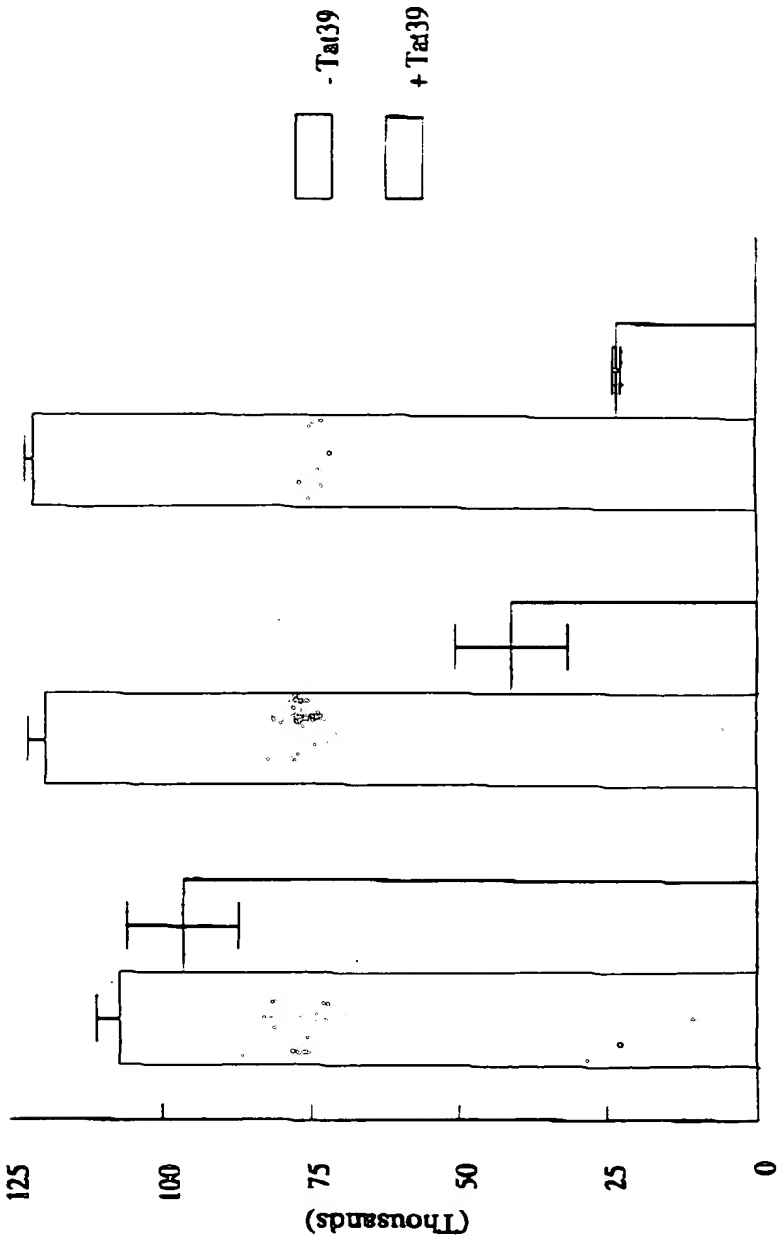


Figure 38B

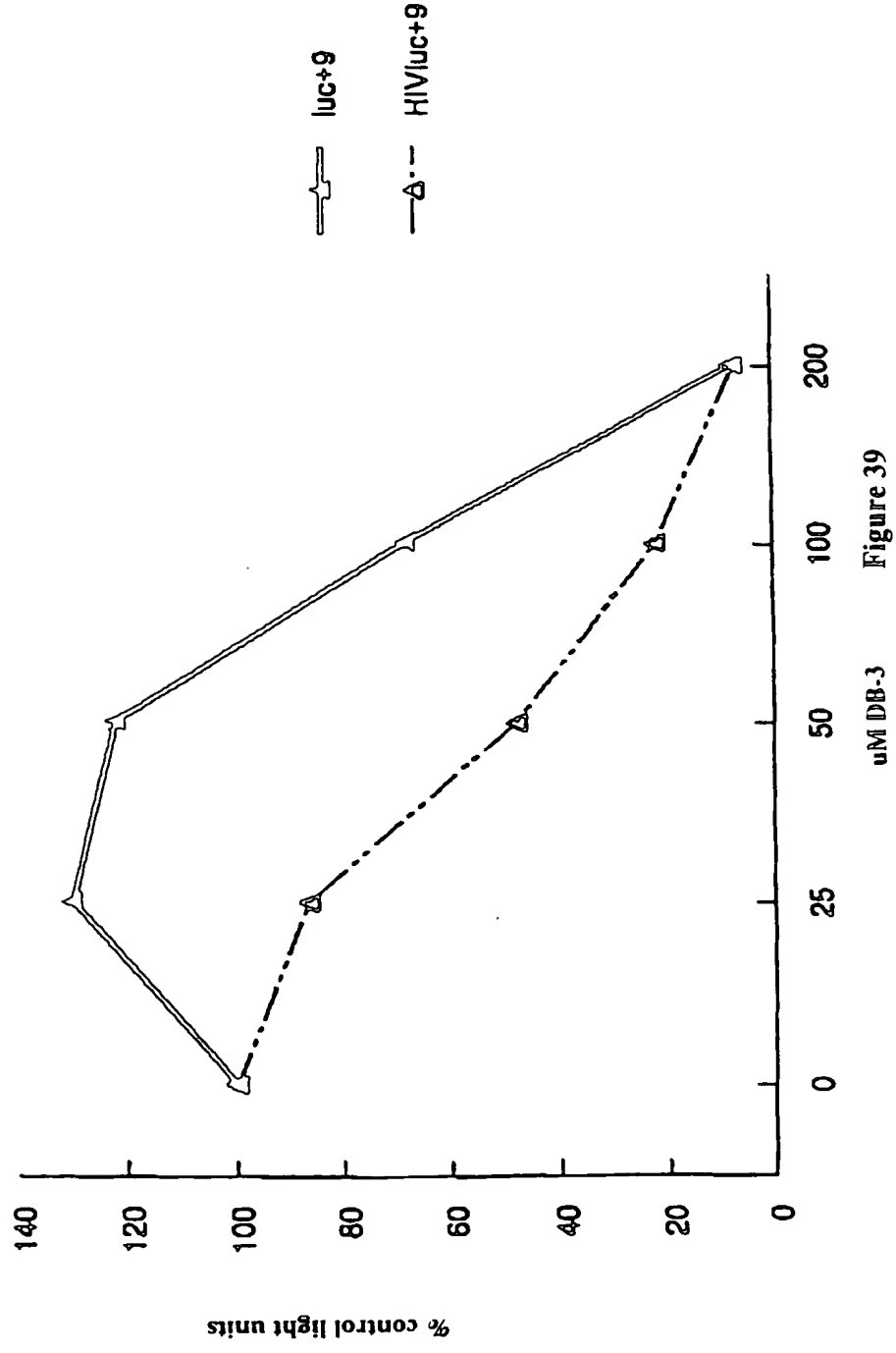

luc+9
HIVluc+9
% control light units
0
20
40
60
80
100
120
140
0
25
50
100
200
uM DB-3

Figure 39

16S A Site rRNA

5'- GGCGUCA CACC U
CCGCUGA GUGG C
A G

Control RNA

5'- GGCGUCACACC U
CCGCAG UGUGG C
A G

Figure 40

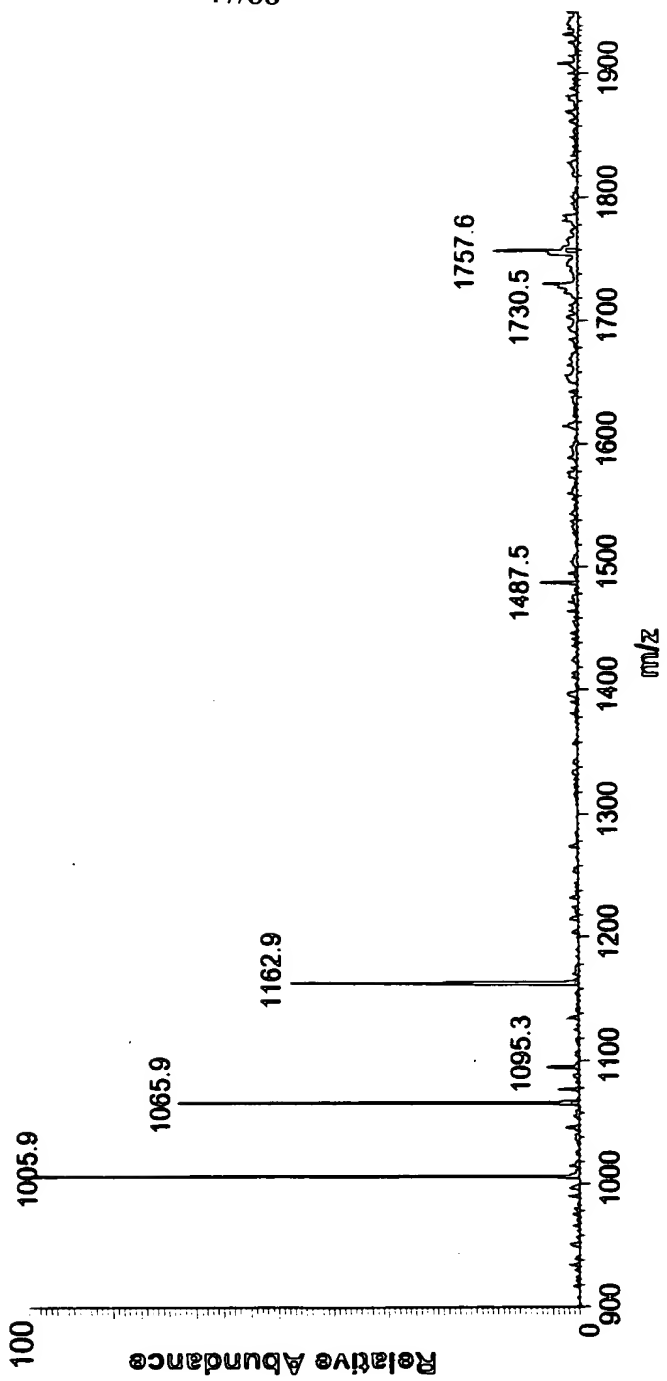

Relative Abundance
100
0
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
m/z
1005.9
1065.9
1095.3
1162.9
1487.5
1730.5
1757.6

Figure 41A

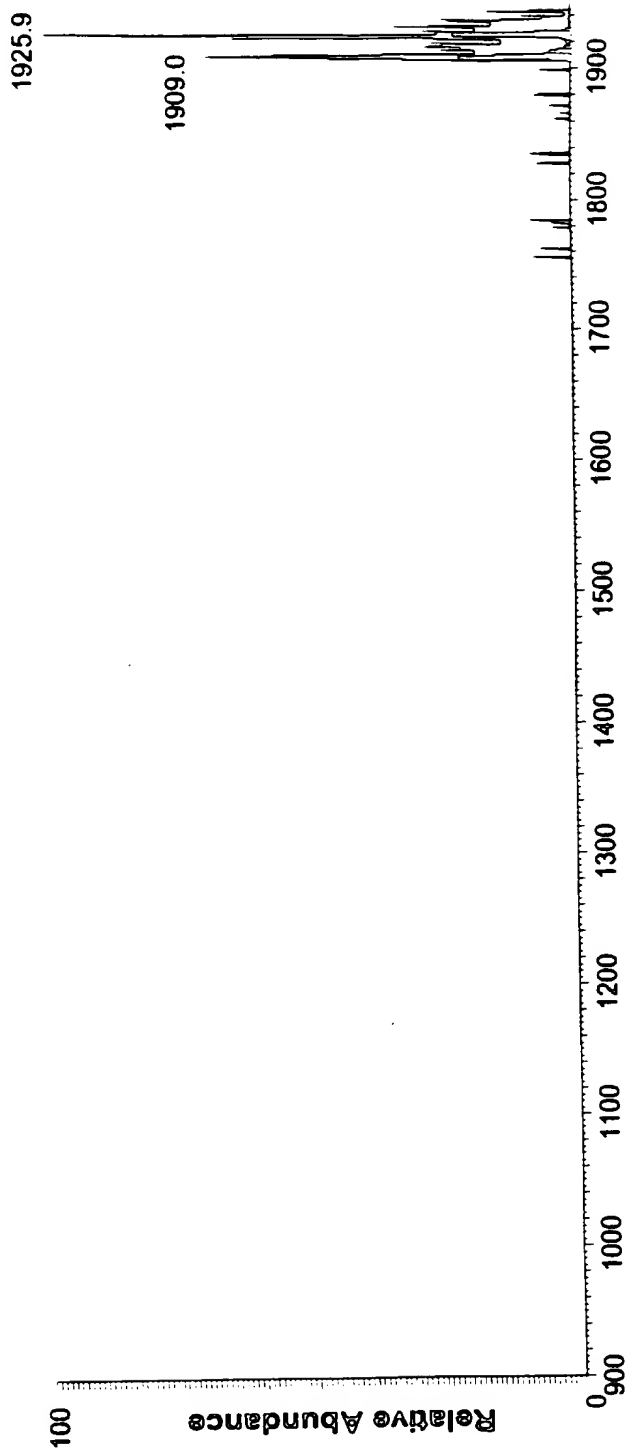

1925.9
1909.0
Relative Abundance
100
0
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
m/z

Figure 41B

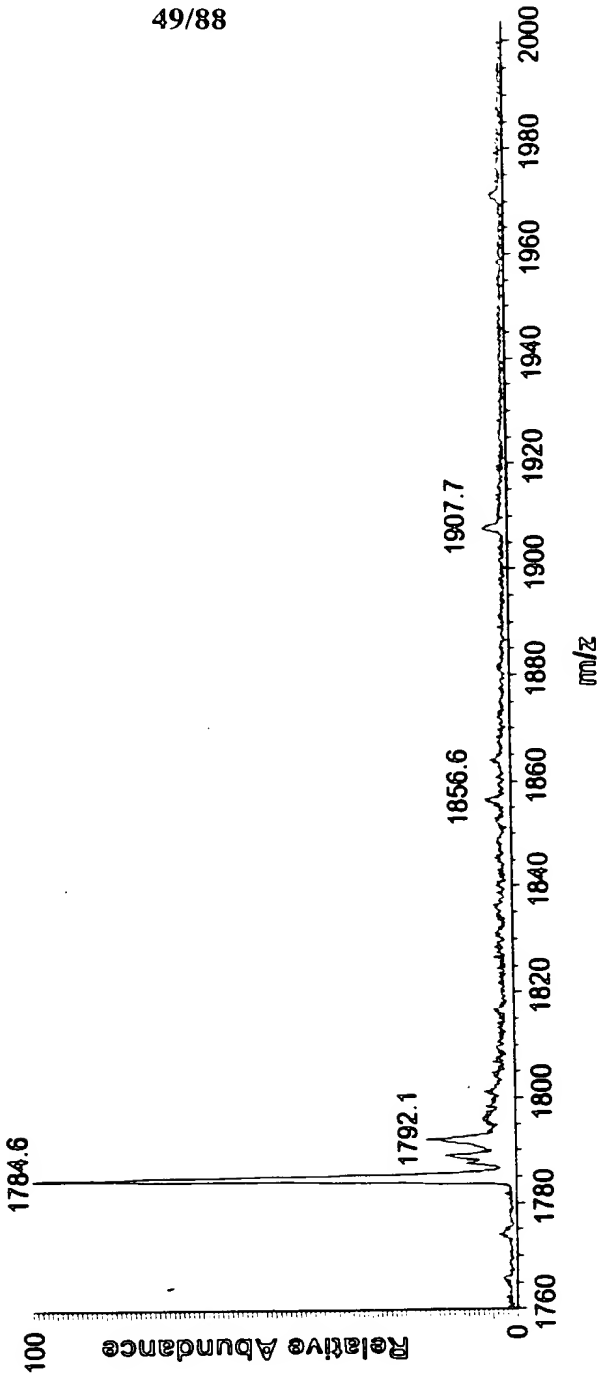

Relative Abundance
100
0
1784.6
1792.1
1856.6
1907.7
1760
1780
1800
1820
1840
1860
1880
1900
1920
1940
1960
1980
2000
m/z

Figure 42A

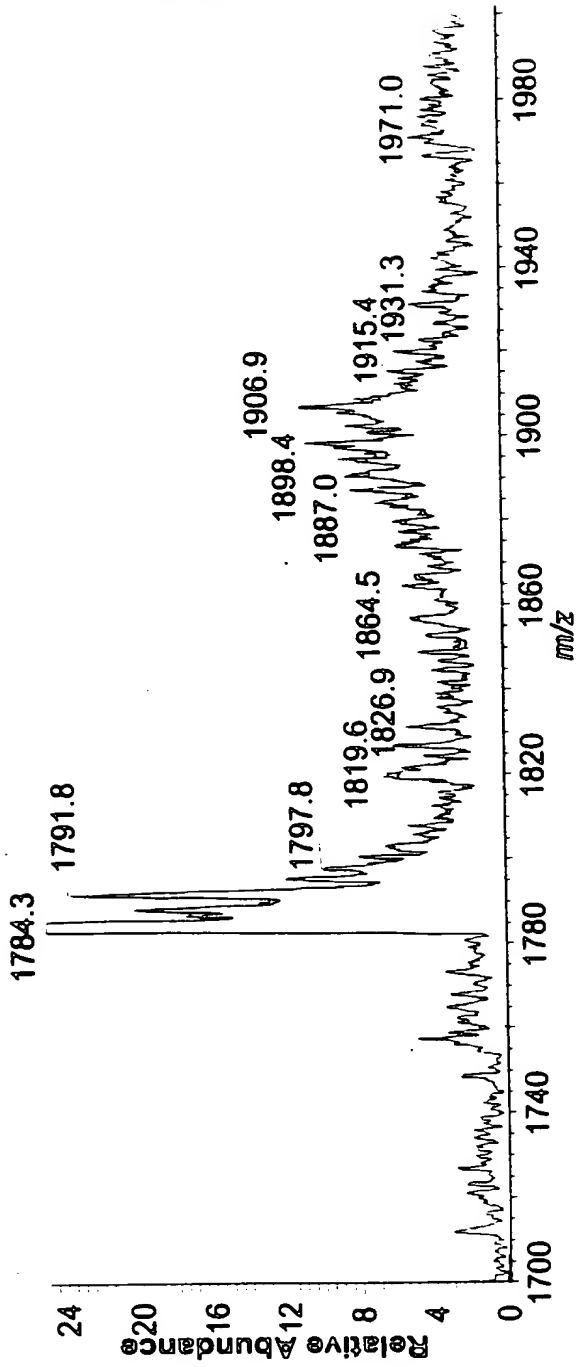

Relative Abundance
m/z
1784.3
1791.8
1797.8
1819.6
1826.9
1864.5
1887.0
1898.4
1906.9
1915.4
1931.3
1971.0
0
4
8
12
16
20
24
1700
1740
1780
1820
1860
1900
1940
1980

Figure 42B

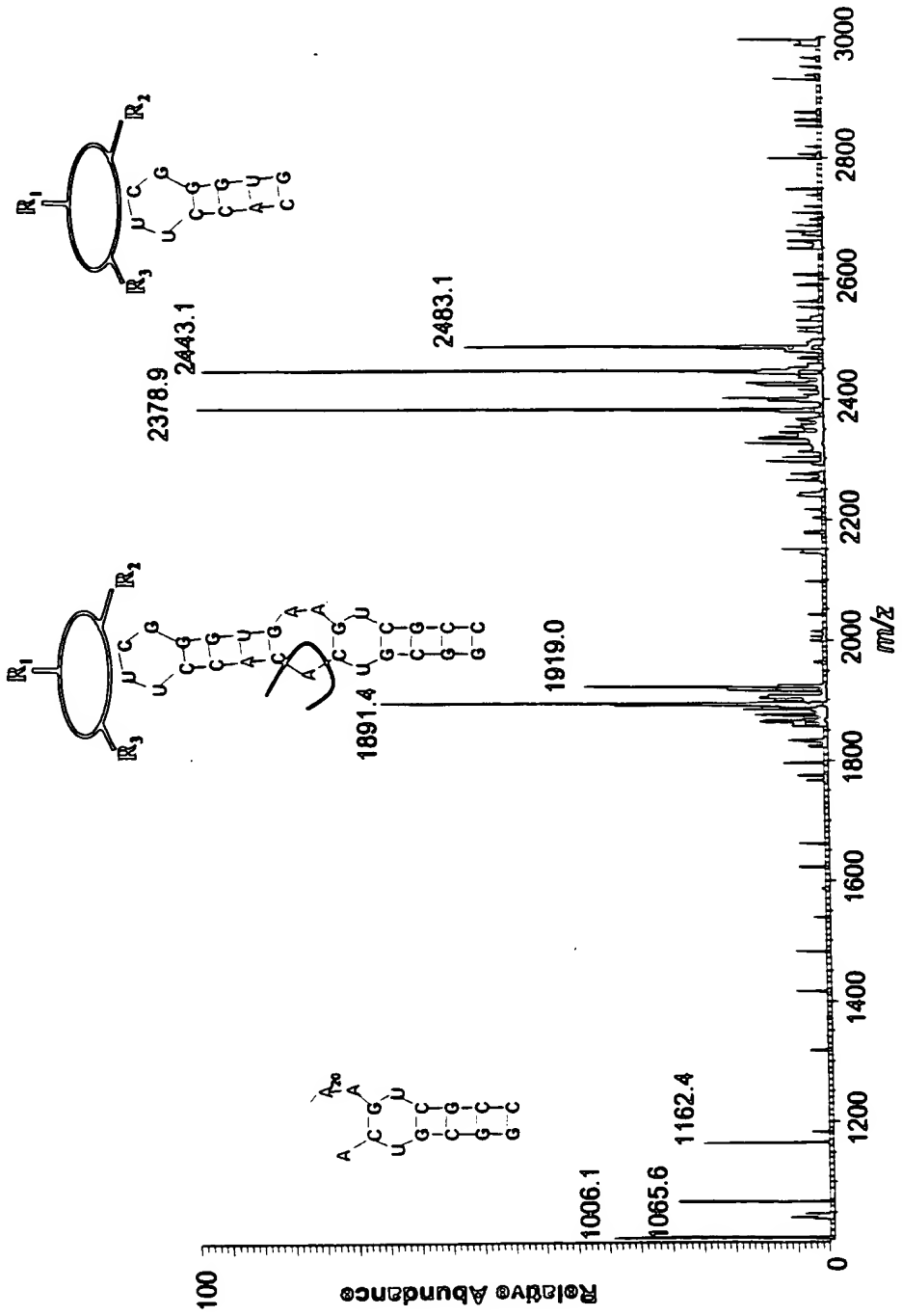

Relative Abundance
m/z
100
0
1200
1400
1600
1800
2000
2200
2400
2600
2800
3000
1006.1
1065.6
1162.4
1891.4
1919.0
2378.9
2443.1
2483.1
R1
R2
R3

Figure 43

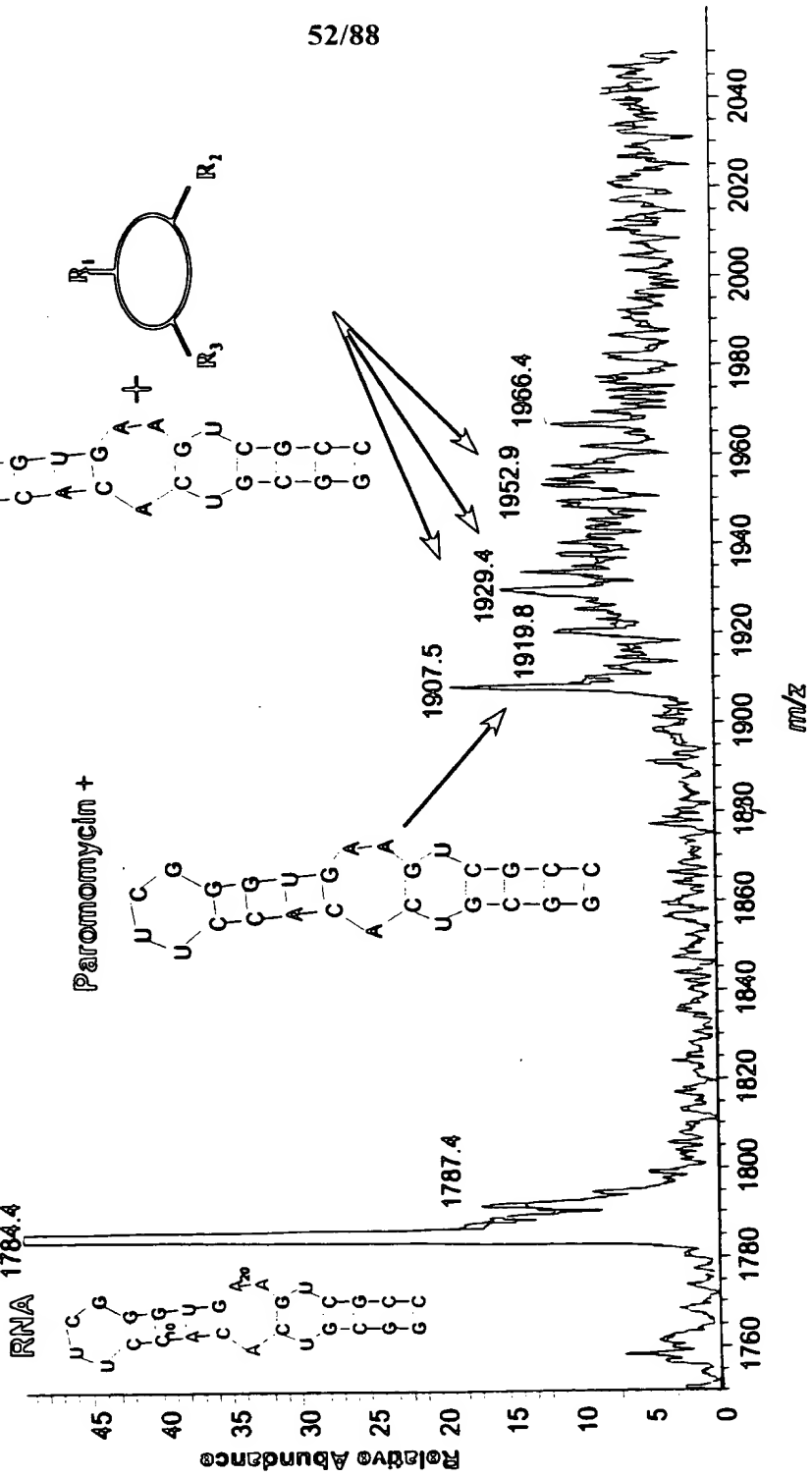

RNA
1784.4
1787.4
Paromomycin +
1907.5
1919.8
1929.4
1952.9
1966.4
Relative Abundance
m/z
0
5
10
15
20
25
30
35
40
45
1760
1780
1800
1820
1840
1860
1880
1900
1920
1940
1960
1980
2000
2020
2040

Figure 44

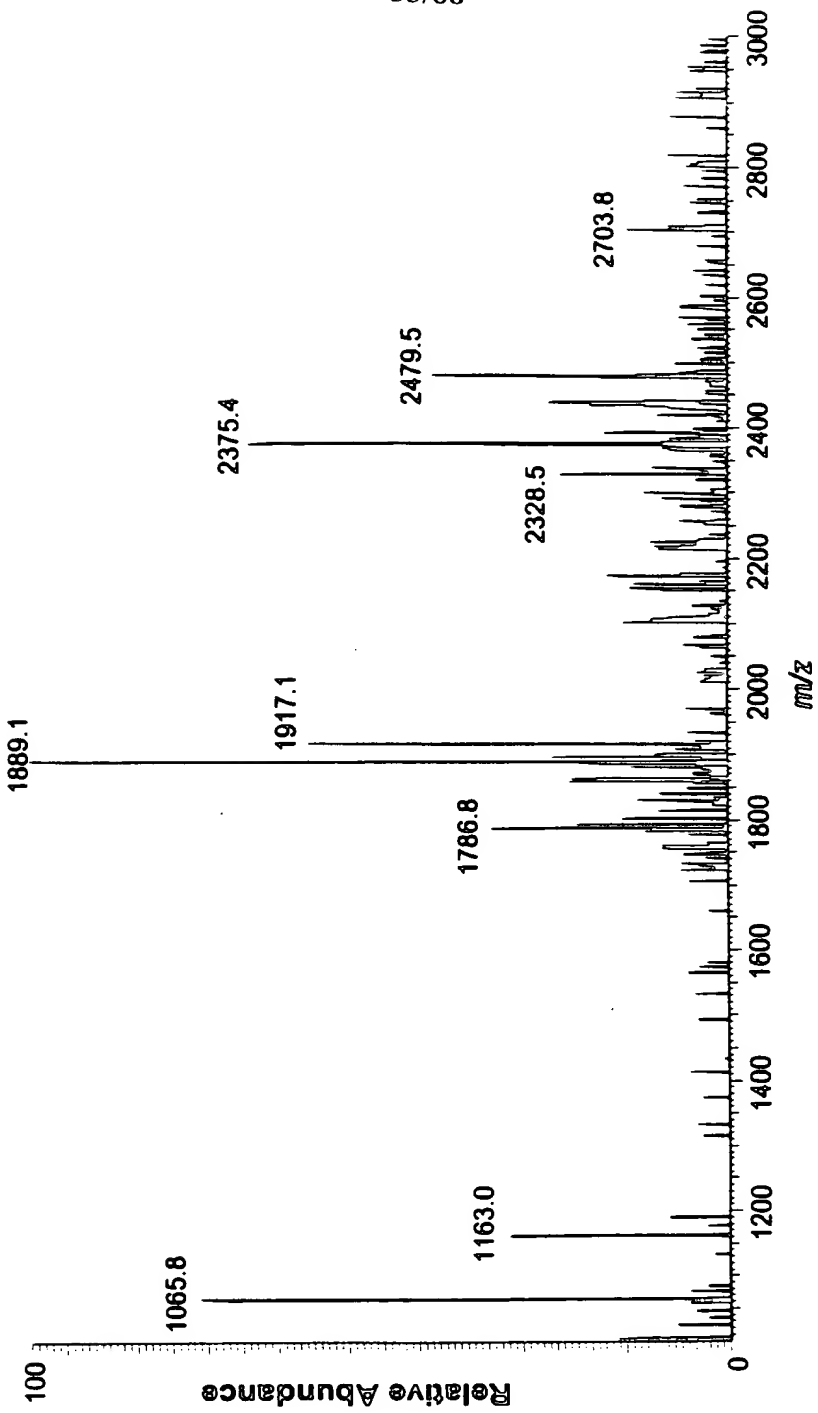

Relative Abundance
100
0
1065.8
1163.0
1786.8
1889.1
1917.1
2328.5
2375.4
2479.5
2703.8
1200
1400
1600
1800
2000
2200
2400
2600
2800
3000
m/z

Figure 45

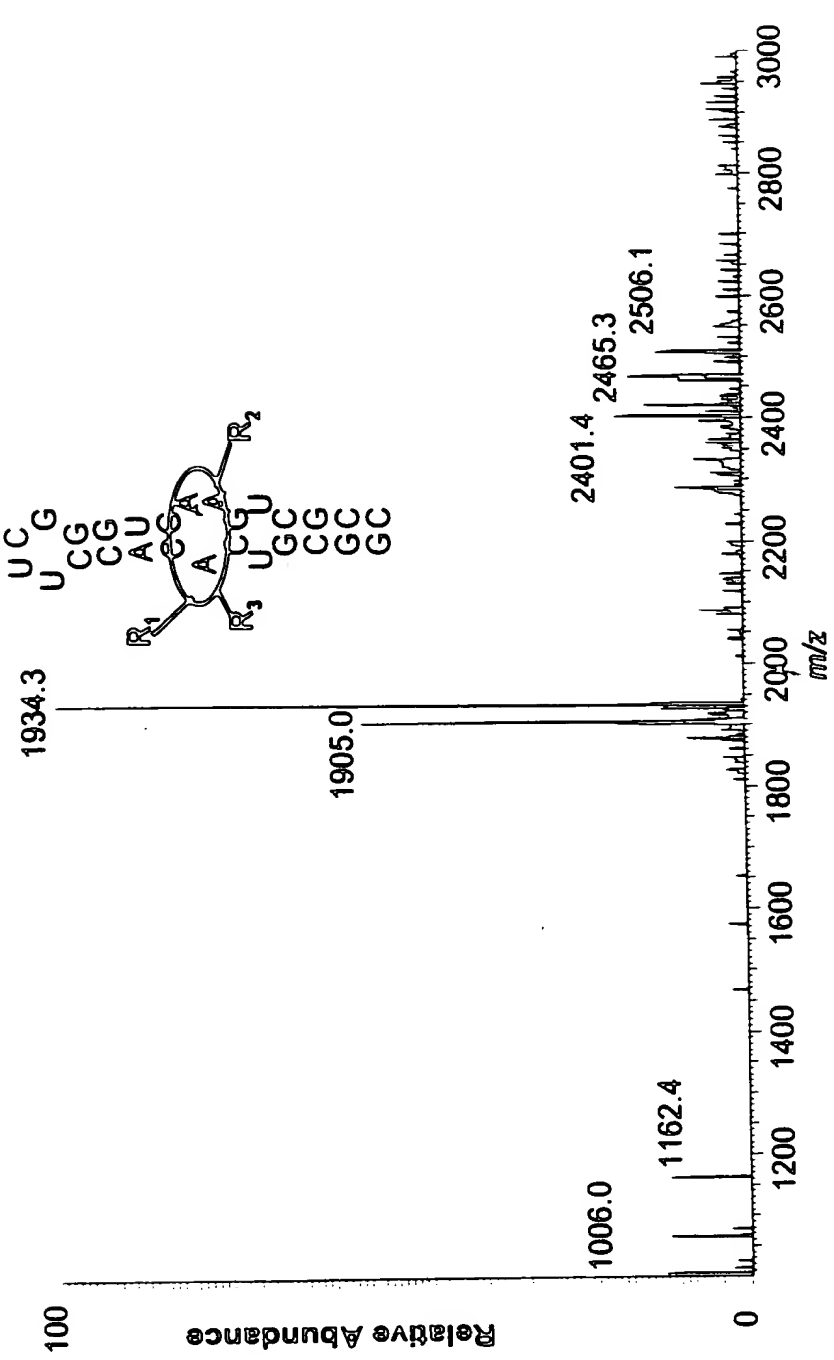

Relative Abundance
100
0
1934.3
1905.0
1006.0
1162.4
2401.4
2465.3
2506.1
1200
1400
1600
1800
2000
2200
2400
2600
2800
3000
m/z
U C
U G
CG
CG
AU
GC
A
A
CG
U
UGC
CG
GC
GC
R1
R2
R3

Figure 46

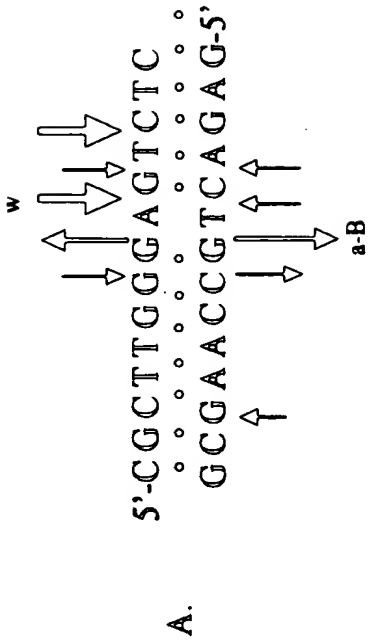

A.
5'-CGCTTGGGAGTCTC
GCGAACCCGTCAGAG-5'
w
a-B
B.
5'-CGCTTGGCAGTCTC
GCGAACCCGTCAGAG-5'

Figure 47

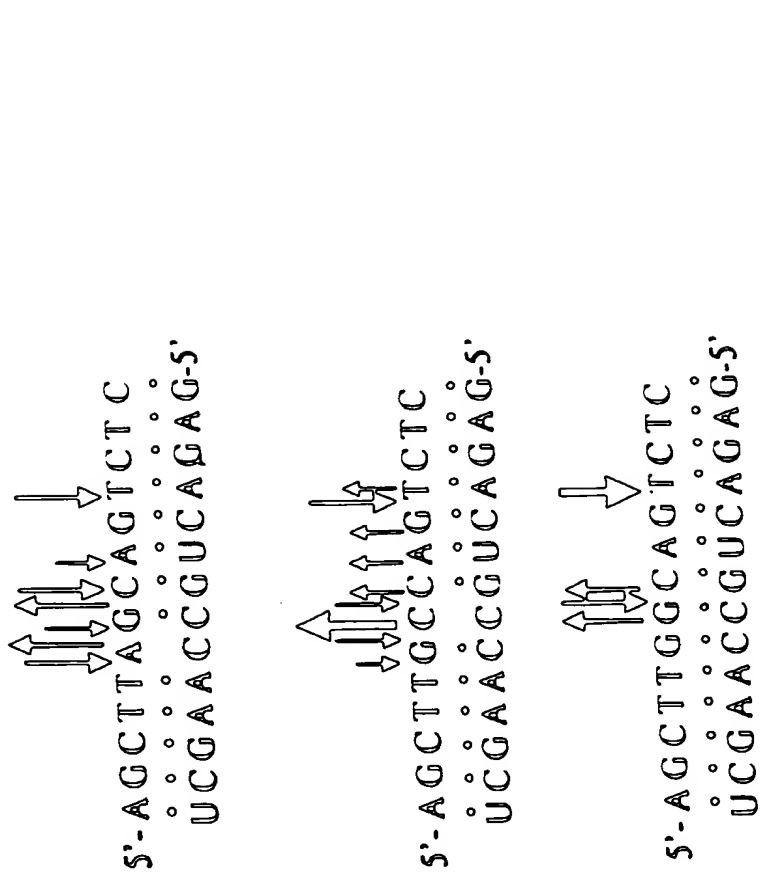


Figure 48 MS Fragmentation of DNA:RNA duplexes

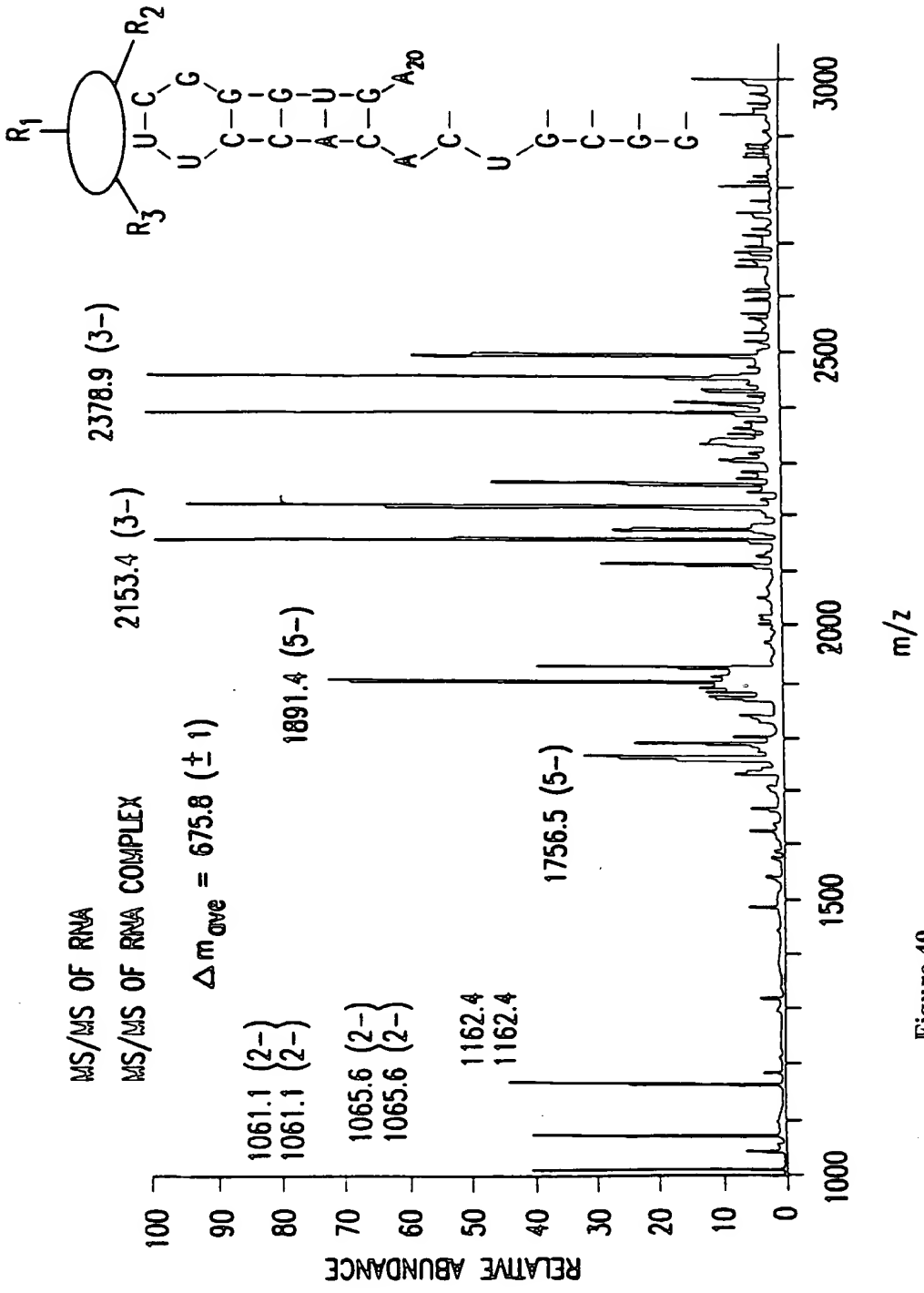

MS/MS OF RNA
MS/MS OF RNA COMPLEX
Δm_{ave} = 675.8 (± 1)
1061.1 (2-)
1061.1 (2-)
1065.6 (2-)
1065.6 (2-)
1162.4
1162.4
1756.5 (5-)
1891.4 (5-)
2153.4 (3-)
2378.9 (3-)
RELATIVE ABUNDANCE
m/z
100
90
80
70
60
50
40
30
20
10
0
1000
1500
2000
2500
3000
R_1
R_2
R_3
A_{20}

Figure 49

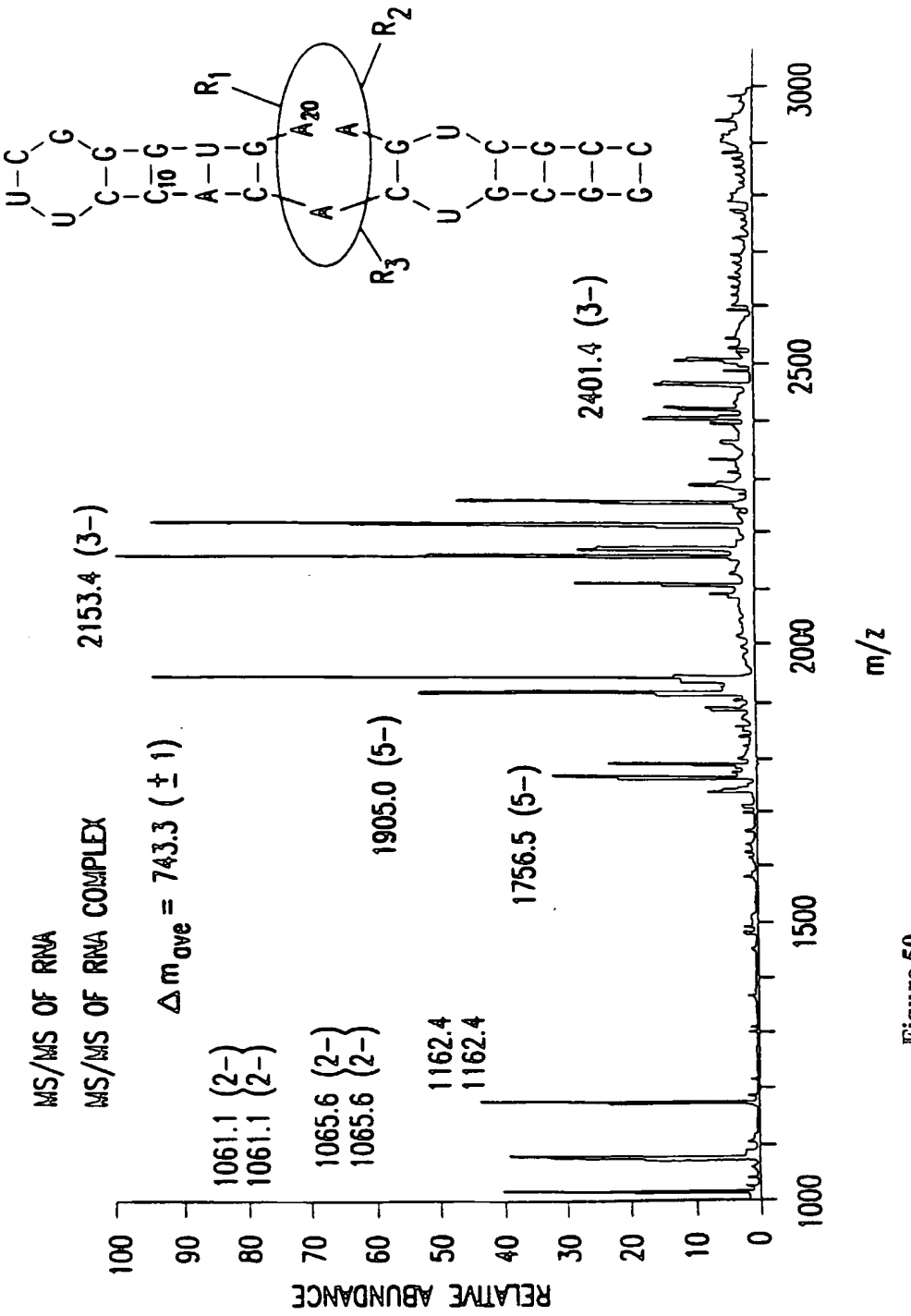

MS/MS OF RNA
MS/MS OF RNA COMPLEX
Δm_{ave} = 743.3 (± 1)
2153.4 (3-)
2401.4 (3-)
1905.0 (5-)
1756.5 (5-)
1061.1 (2-)
1061.1 (2-)
1065.6 (2-)
1065.6 (2-)
1162.4
1162.4
RELATIVE ABUNDANCE
m/z
100
90
80
70
60
50
40
30
20
10
0
1000
1500
2000
2500
3000
R_1
R_2
R_3
A_{20}
C_{10}

Figure 50

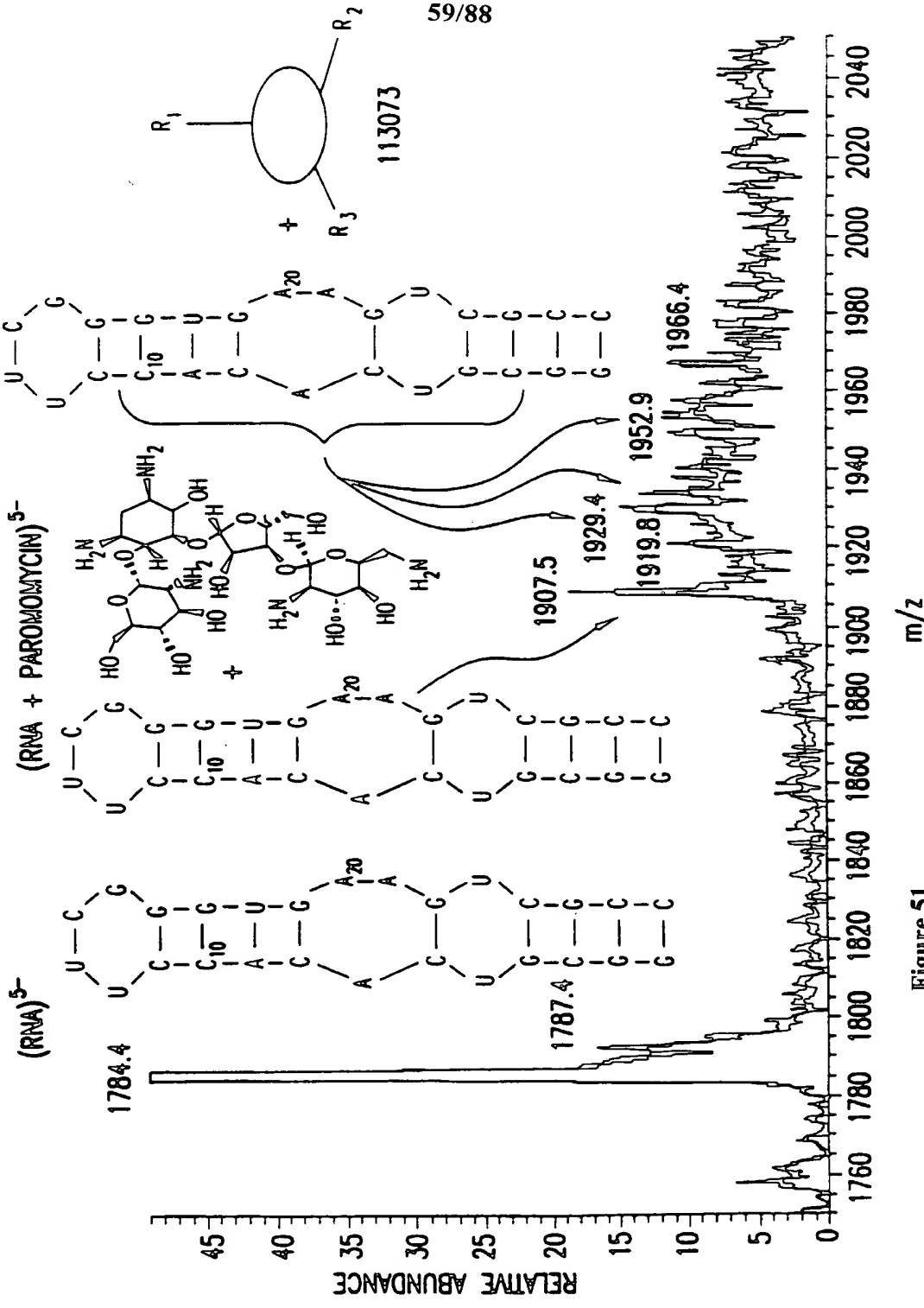

RELATIVE ABUNDANCE
m/z
(RNA)5-
(RNA + PAROMOMYCIN)5-
113073
1784.4
1787.4
1907.5
1919.8
1929.4
1952.9
1966.4

Figure 51

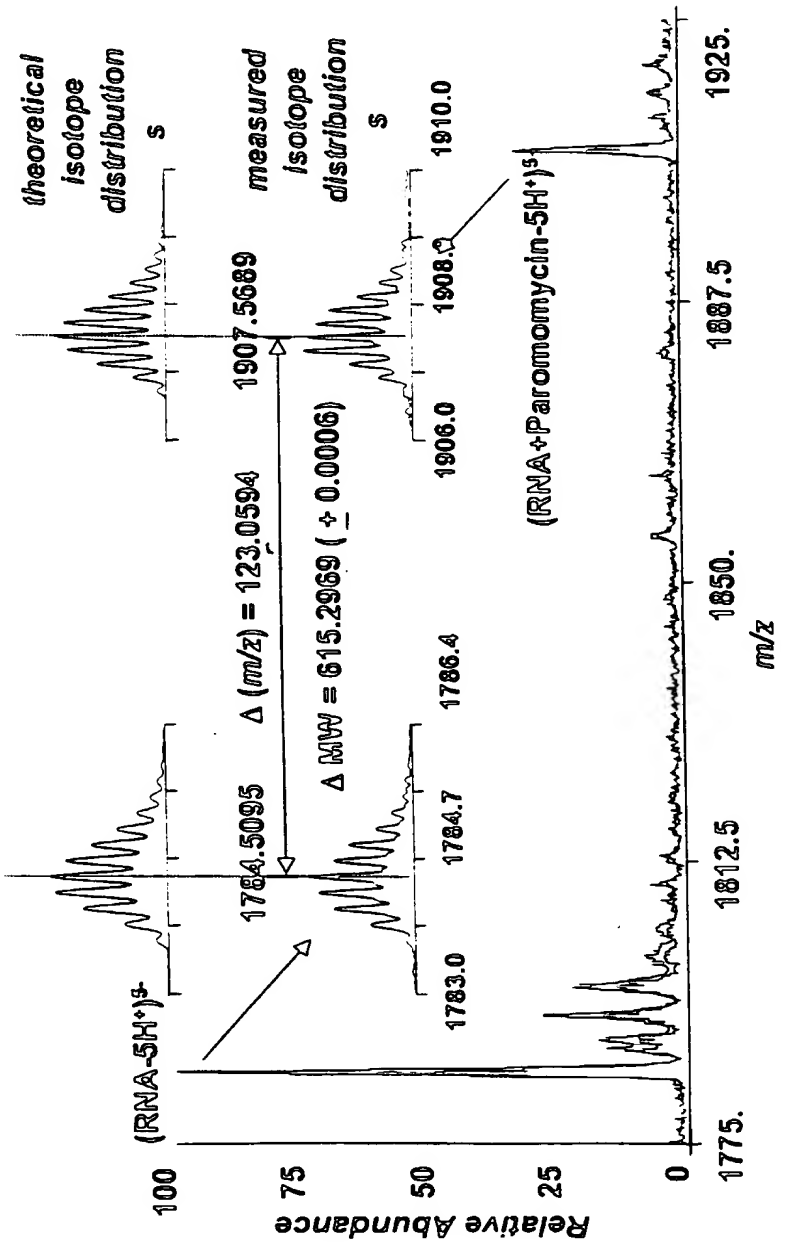

theoretical isotope distribution
measured isotope distribution
(RNA-5H+)5-
(RNA+Paromomycin-5H+)5-
1784.5095
Δ (m/z) = 123.0594
1907.5689
Δ MW = 615.2969 (± 0.0006)
1783.0
1784.7
1786.4
1906.0
1908.2
1910.0
Relative Abundance
100
75
50
25
0
1775.
1812.5
1850.
1887.5
1925.
m/z

Figure 52

Figure 53

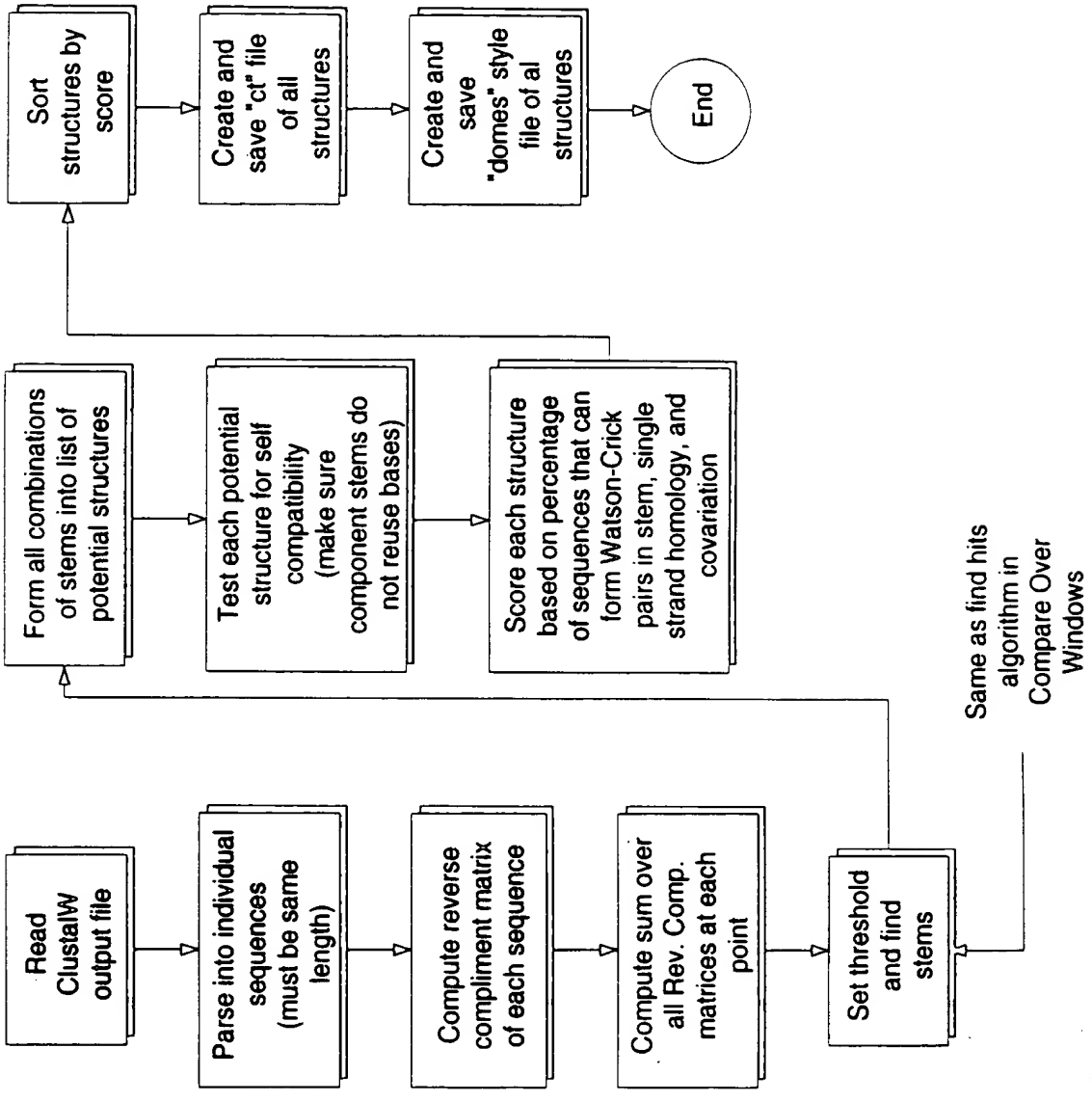

Read ClustalW output file
Parse into individual sequences (must be same length)
Compute reverse compliment matrix of each sequence
Compute sum over all Rev. Comp. matrices at each point
Set threshold and find stems
Same as find hits algorithm in Compare Over Windows
Form all combinations of stems into list of potential structures
Test each potential structure for self compatibility (make sure component stems do not reuse bases)
Score each structure based on percentage of sequences that can form Watson-Crick pairs in stem, single strand homology, and covariation
Sort structures by score
Create and save "ct" file of all structures
Create and save "domes" style file of al structures
End

Figure 54

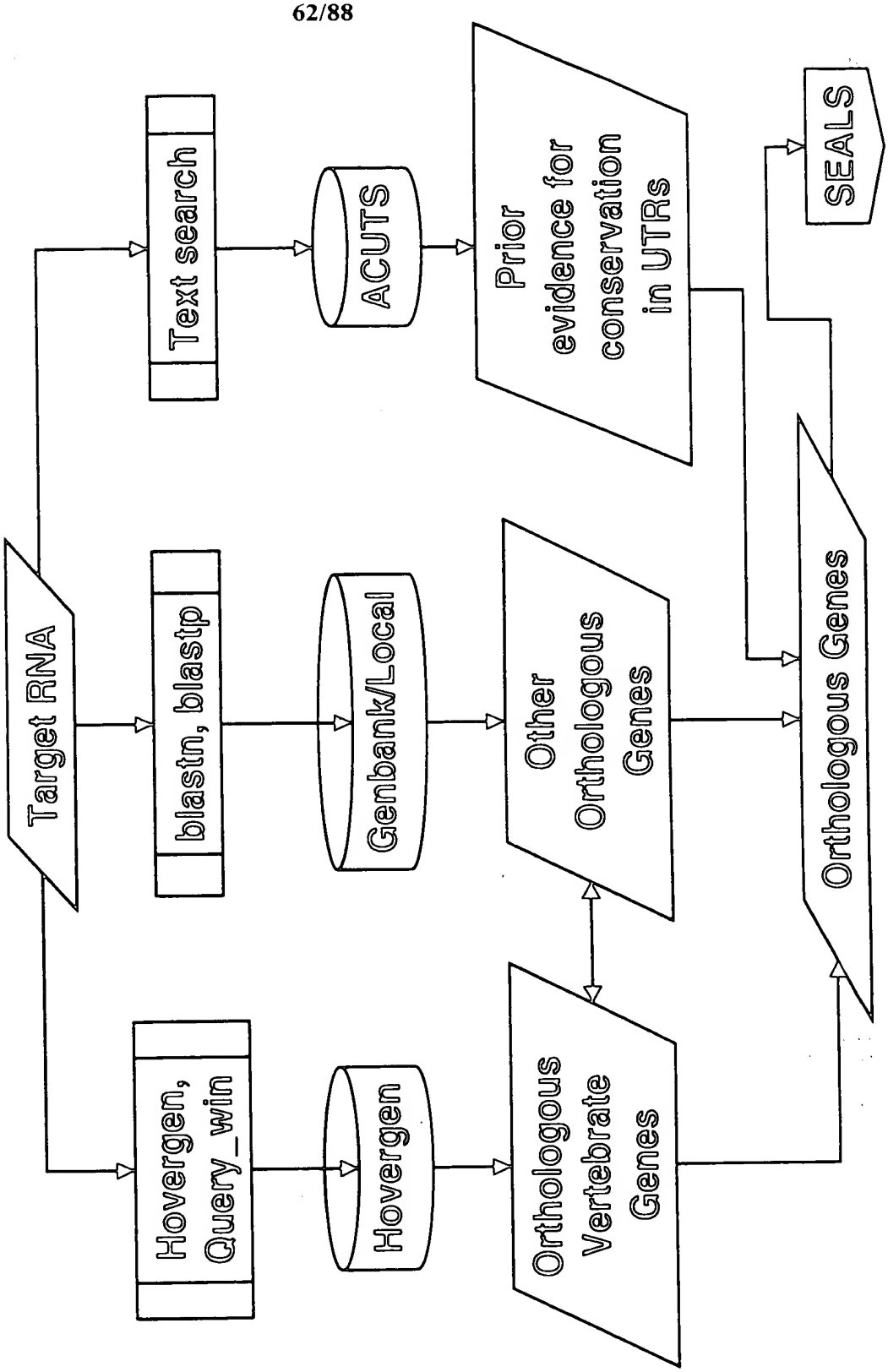

Target RNA
blastn, blastp
Text search
Genbank/Local
ACUTS
Other Orthologous Genes
Prior evidence for conservation in UTRs
SEALS
Orthologous Genes
Hovergen, Query_win
Hovergen
Orthologous Vertebrate Genes

Figure 55

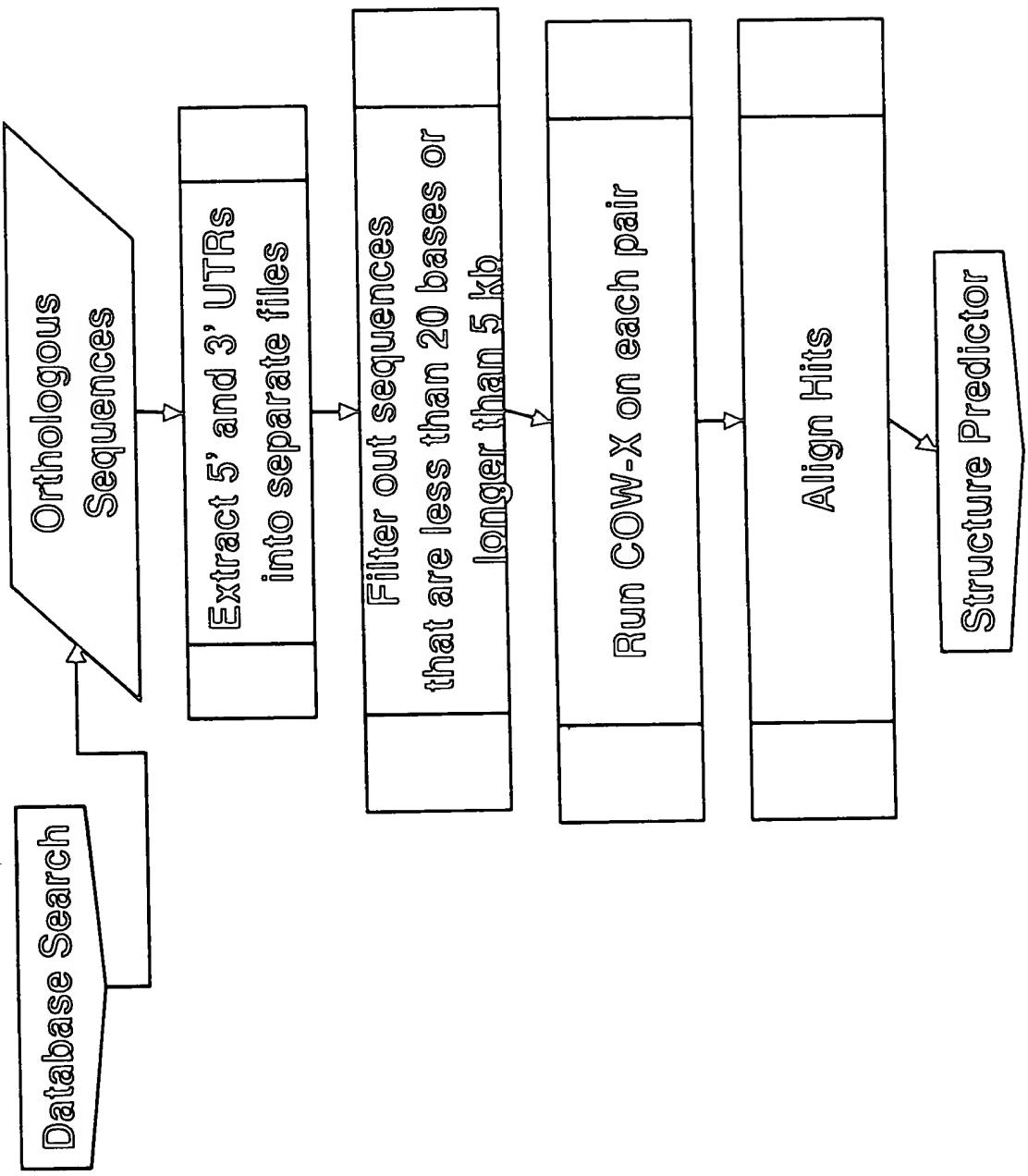

Orthologous Sequences
Database Search
Extract 5' and 3' UTRs into separate files
Filter out sequences that are less than 20 bases or longer than 5 kb
Run COW-X on each pair
Align Hits
Structure Predictor

Figure 56

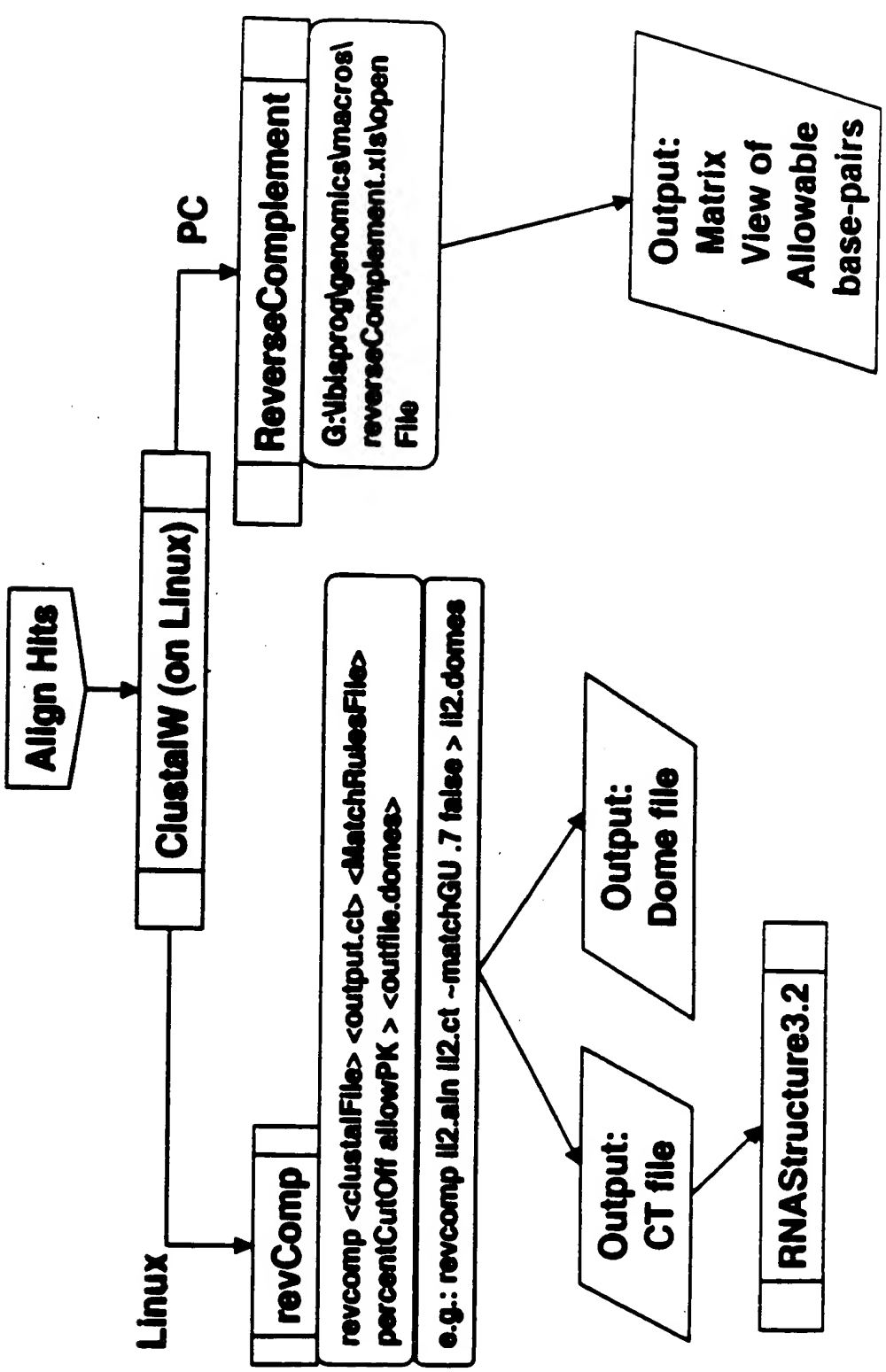

Linux
PC
Align Hits
ClustalW (on Linux)
ReverseComplement
G:\Ibisprog\genomics\macros\reverseComplement.xls\open File
Output: Matrix View of Allowable base-pairs
revComp
revcomp <clustalFile> <output.ct> <MatchRulesFile> percentCutOff allowPK > <outfile.domes>
e.g.: revcomp ll2.aln ll2.ct ~matchGU .7 false > ll2.domes
Output: Dome file
Output: CT file
RNAStructure3.2

Figure 57

Score: 165.0

NNUNNNNGAUNCUUUNNGUAAGCCCNANGNGNNNNAAANNNNNN	Consensus
-UUUUAAGAUUCUUUUUGUAAGCCCUACGGGCU-UAAAAAUUCA	gi|163204|M12791
-UUUUAAGAUUCUUUUUGUAAGCCCUAGGCGUGCUAAAAACUC-	gi|304313|L19402
-UUUAAAGAUUCUUUUUGUAAGCCCCAAGGGCUCAAAAAUGUU-	gi|198302|K02797
-UUUAAAGAUUCUUUUUGUAAGCCCCAAGGGCUCAAAAAUGUU-	gi|204909|M22899
CUUUUAAGAUUCUUUU-GUAAGCCCUAAGGGCUCUAAAAUGGU-	gi|3450973|AF082496
-UUUUAUGAUUCUUUUUGUAAGCCCUAGGGGCUCUAAAAUGGU-	gi|33808|X01586
-AUAUUUGAUCCUUUCUGUAAGCCCUACGGGCUCAAAAUGUAC-	gi|3087784|AJ224516

66/88

Figure 58

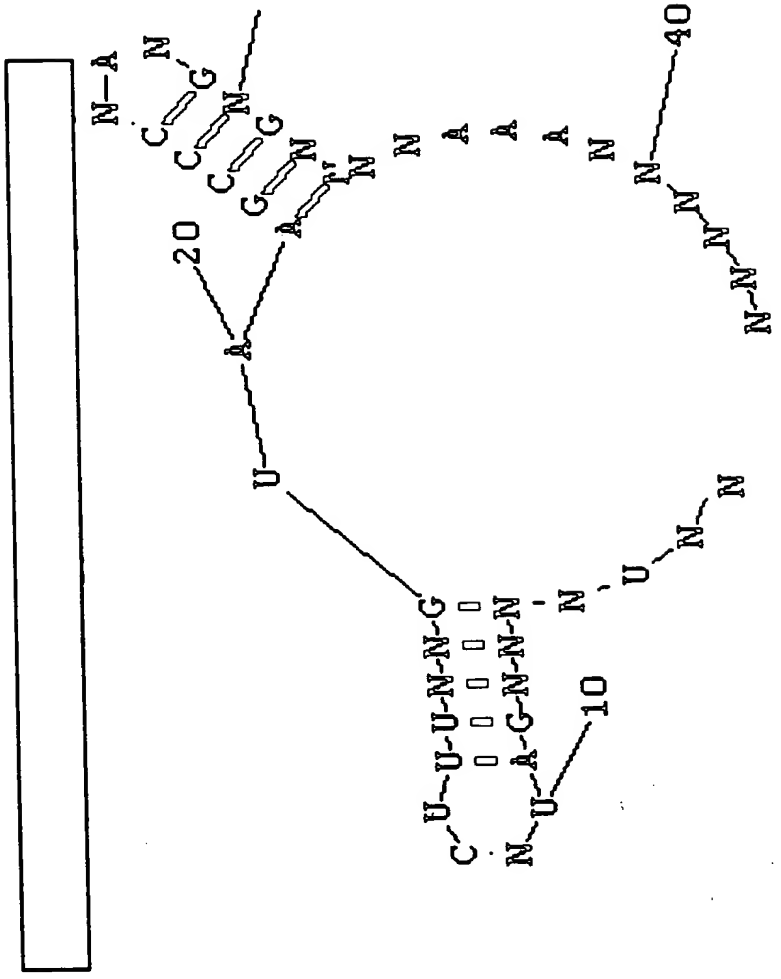


Figure 59

MASS of 60-Member Ibis Library Against 16S A-site RNA

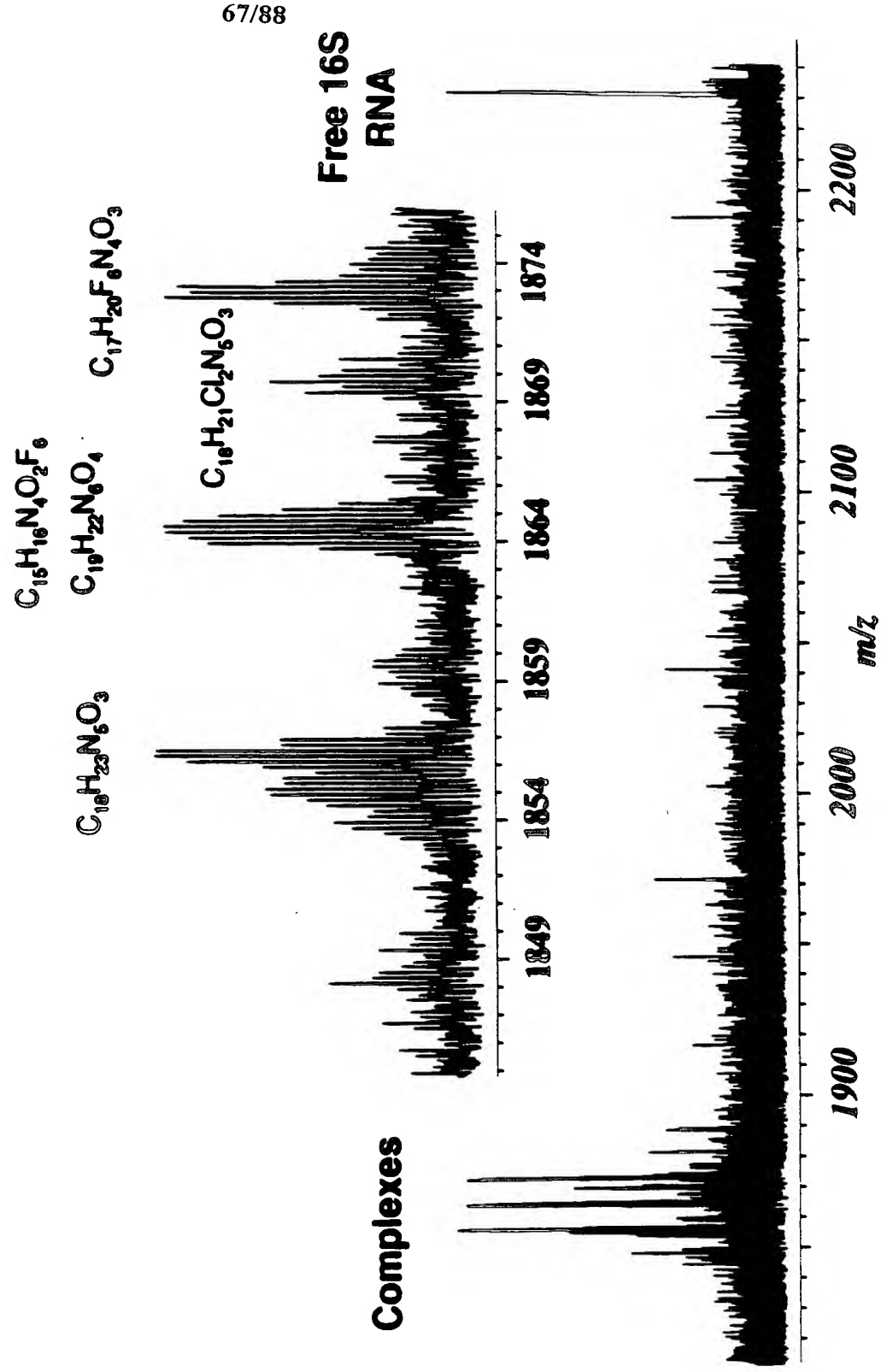


Figure 60

MASS of 60-member Library against 16S A-site Model

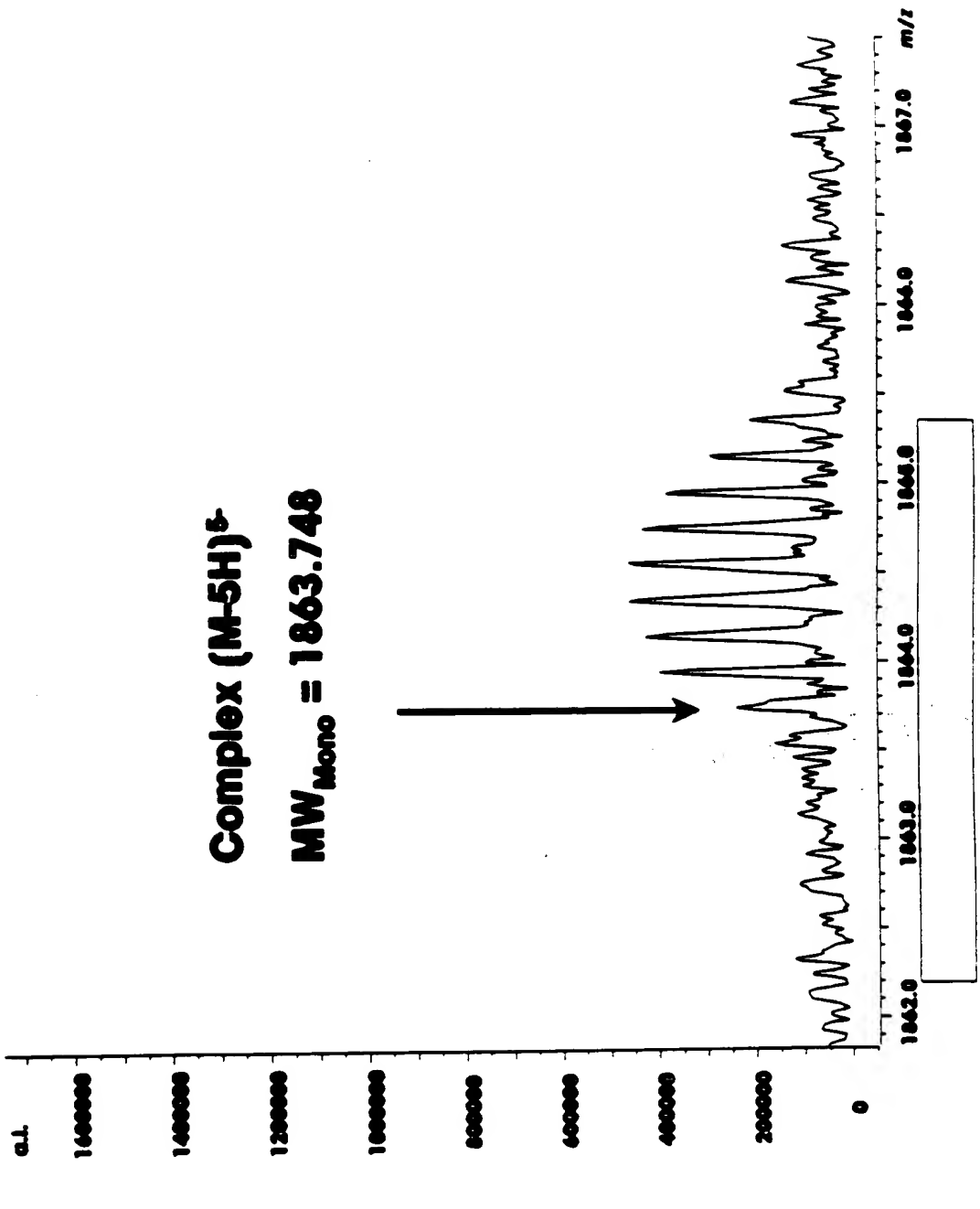


Figure 61

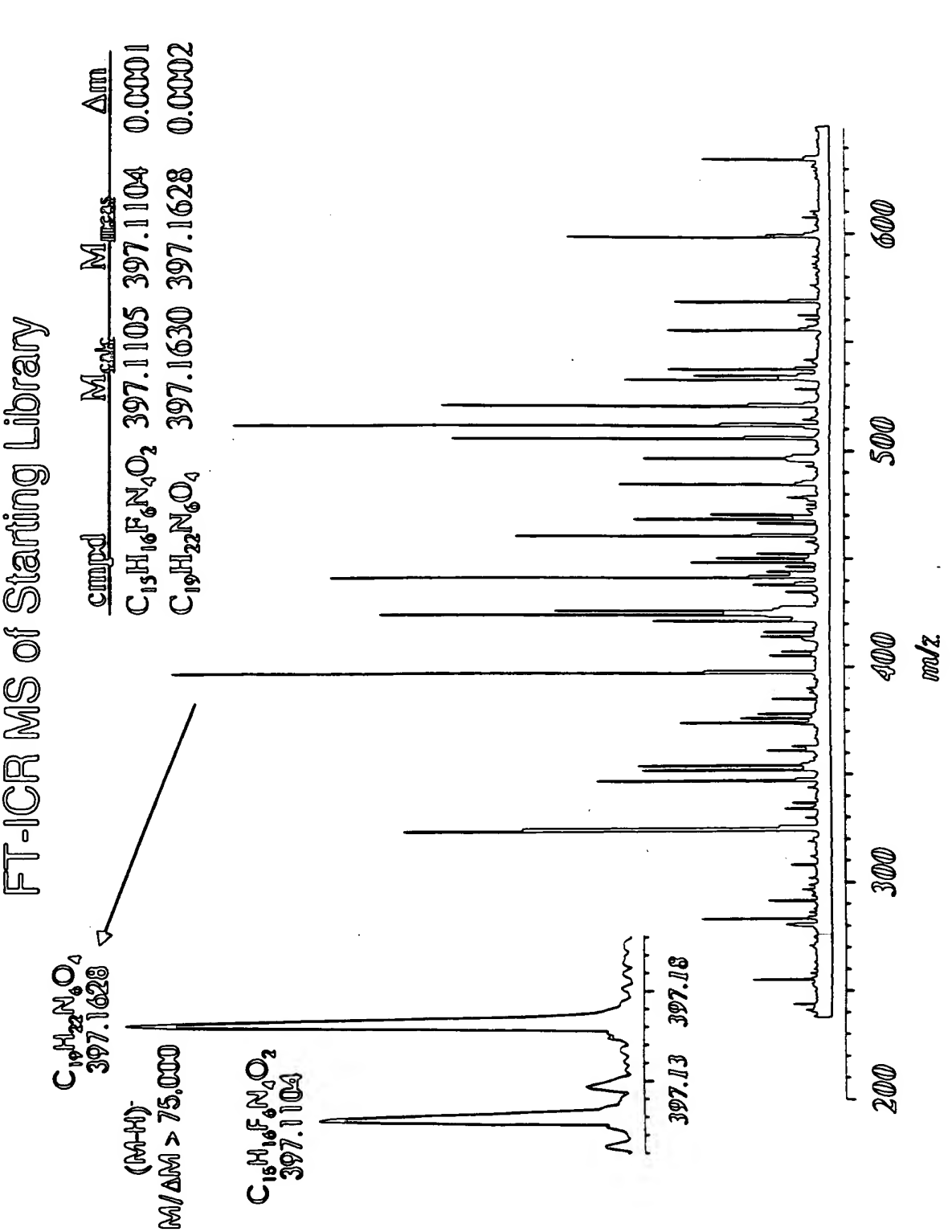

FT-ICR MS of Starting Library
cmpd M_{calc} M_{meas} Δm
$C_{13}H_{16}F_6N_4O_2$ 397.1105 397.1104 0.0001
$C_{19}H_{22}N_6O_4$ 397.1630 397.1628 0.0002
$C_{19}H_{22}N_6O_4$
397.1628
(M-H)-
M/ΔM > 75,000
$C_{15}H_{16}F_6N_4O_2$
397.1104
397.13 397.18
200 300 400 500 600
m/z

Figure 62

Compound Identification from a 60-member Combinatorial library with MASS

Complex M_{meas}	**9320.300±.009 Da**
RNA M_{meas}	**8922.189±.009**
ΔM	**398.111±.009 Da**

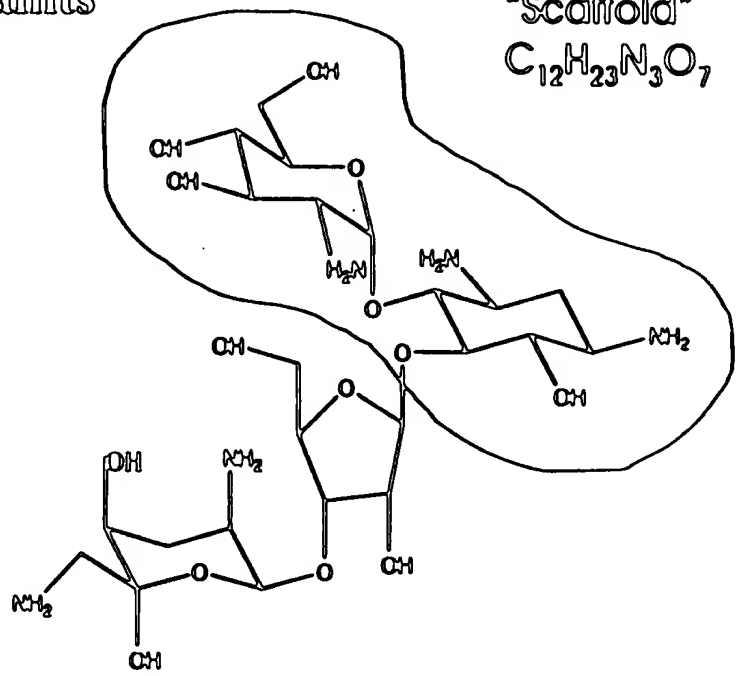
$C_{15}H_{16}N_4O_2F_6$	**398.117 Da**
~~$C_{19}H_{22}N_6O_4$~~	**~~398.163~~**

Docket No.: IBIS-0171 Appln No. 09/310,844
Title: MOLECULAR INTERACTION SITES OF INTERLEUKIN-2 RNA AND METHODS OF MODULATING THE SAME
Inventor(s): David J. Ecker, Richard Griffey, Stanley T. Crooke, Ranga Sampath, Eric Swayze, Venkataraman Mohan, Steven Hofstadler, John McNeil
Paul K. Legaard 215-568-3100
Sheet 70 of 88

Figure 63

Elemental Composition Constraints

"Scaffold"
$C_{12}H_{23}N_3O_7$



Measured Mass: 615.2969
Mass Tolerance: 1.0 ppm
Charge: 0

Element	Min. atoms	Max. atoms
^{12}C	12	30
^{1}H	23	60
^{16}O	7	20
^{14}N	3	20

Possible Elemental Compositions:

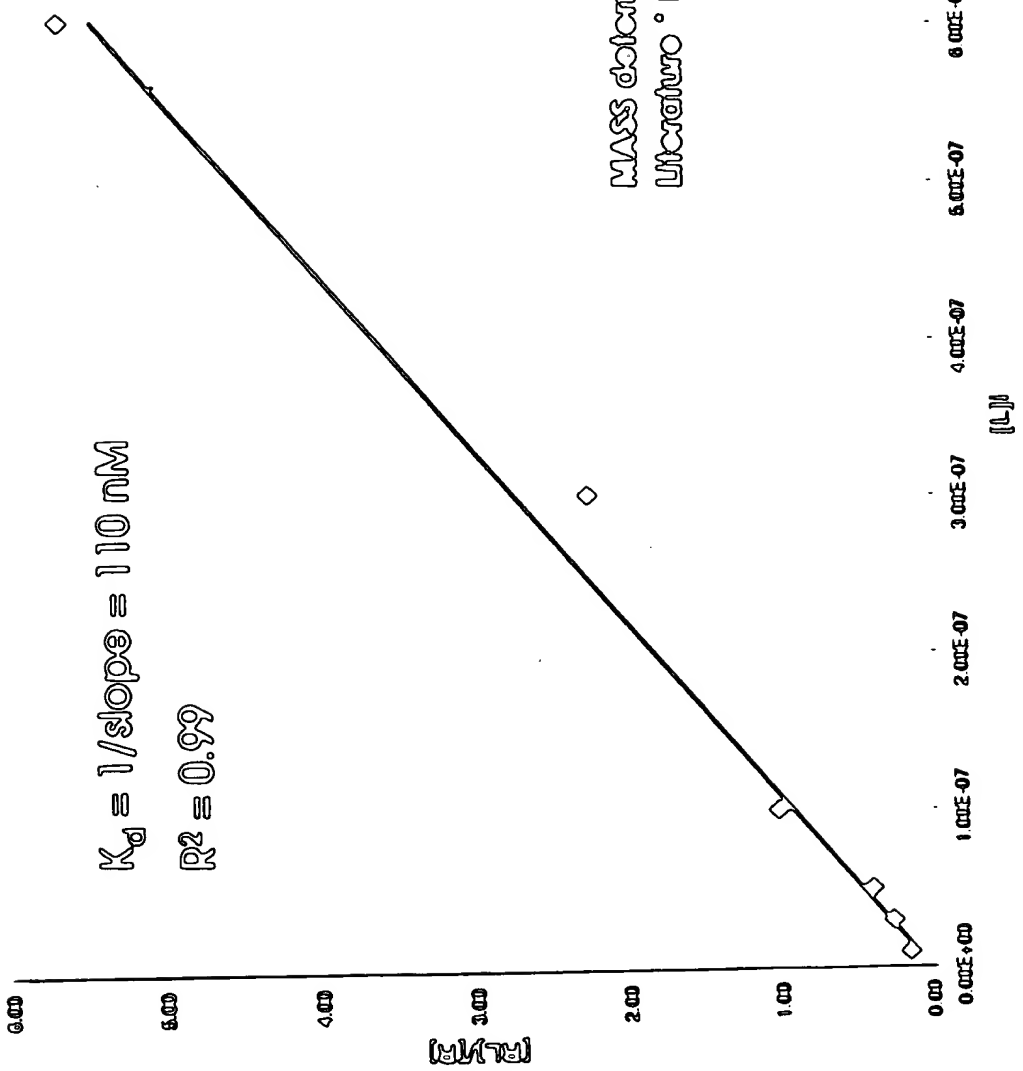
Calc. Mass (amu)	Error (ppm)	Molecular Formula
~~615.296291~~	~~0.98~~	~~$^{16}O_4\ ^{14}N_{19}\ ^{12}C_{21}\ ^{1}H_{33}$~~
615.296298	0.98	$^{16}O_9\ ^{14}N_{12}\ ^{12}C_{22}\ ^{1}H_{39}$
615.296305	0.97	$^{16}O_{14}\ ^{14}N_5\ ^{12}C_{23}\ ^{1}H_{45}$
~~615.296808~~	~~0.15~~	~~$^{16}O_{15}\ ^{14}N_{17}\ ^{12}C_8\ ^{1}H_{41}$~~
~~615.296815~~	~~0.14~~	~~$^{16}O_{20}\ ^{14}N_{10}\ ^{12}C_9\ ^{1}H_{47}$~~

Further constrain by elemental composition of "letters"

unintended products...

Figure 64

MASS K_d determination for 16S-Paromomycin



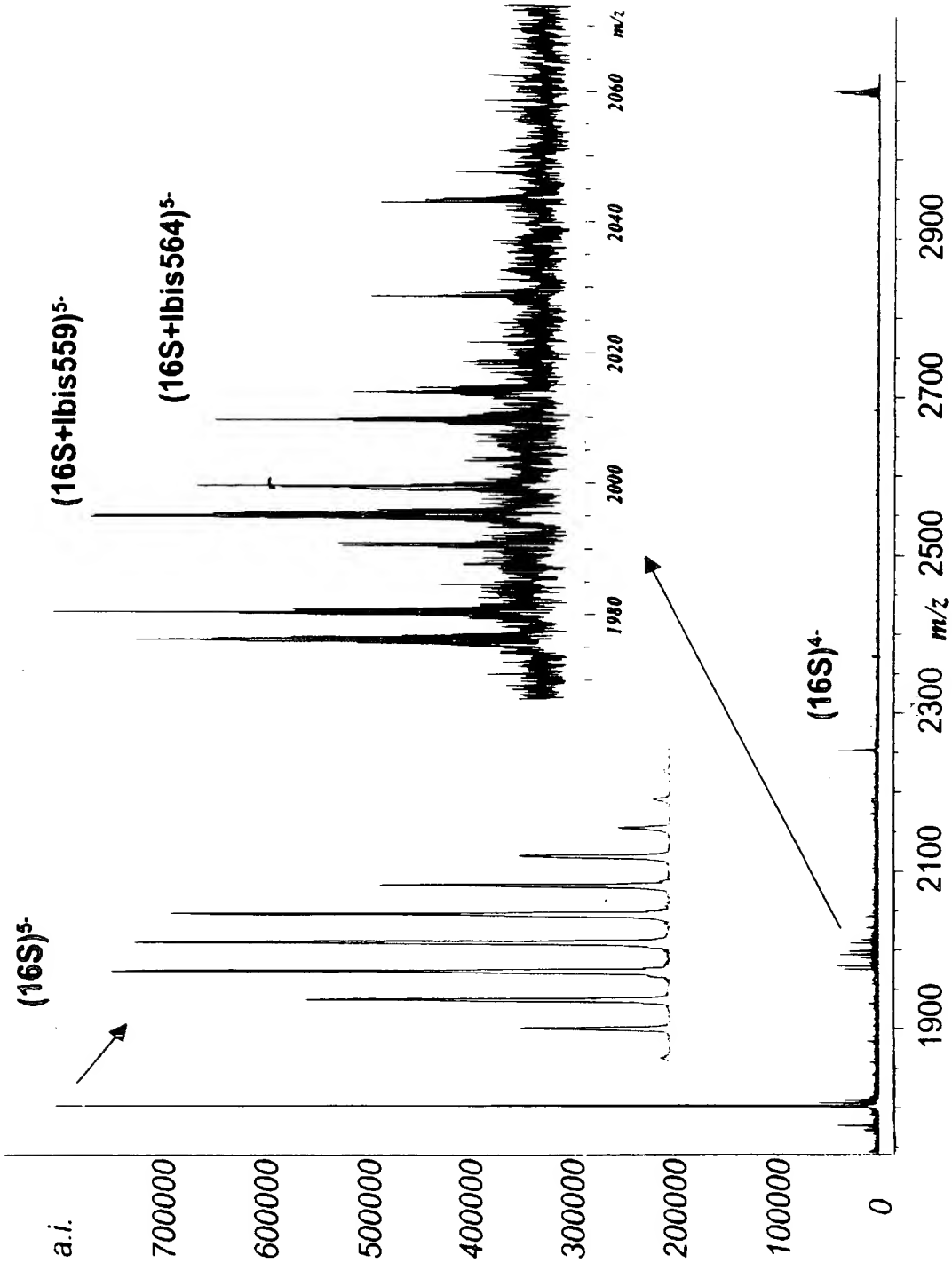

(16S)5-
(16S+Ibis559)5-
(16S+Ibis564)5-
(16S)4-
a.i.
700000
600000
500000
400000
300000
200000
100000
0
1900
2100
2300
2500
2700
2900
m/z
1980
2000
2020
2040
2060
m/z

Figure 65

Figure 66

MASS Protection Assay

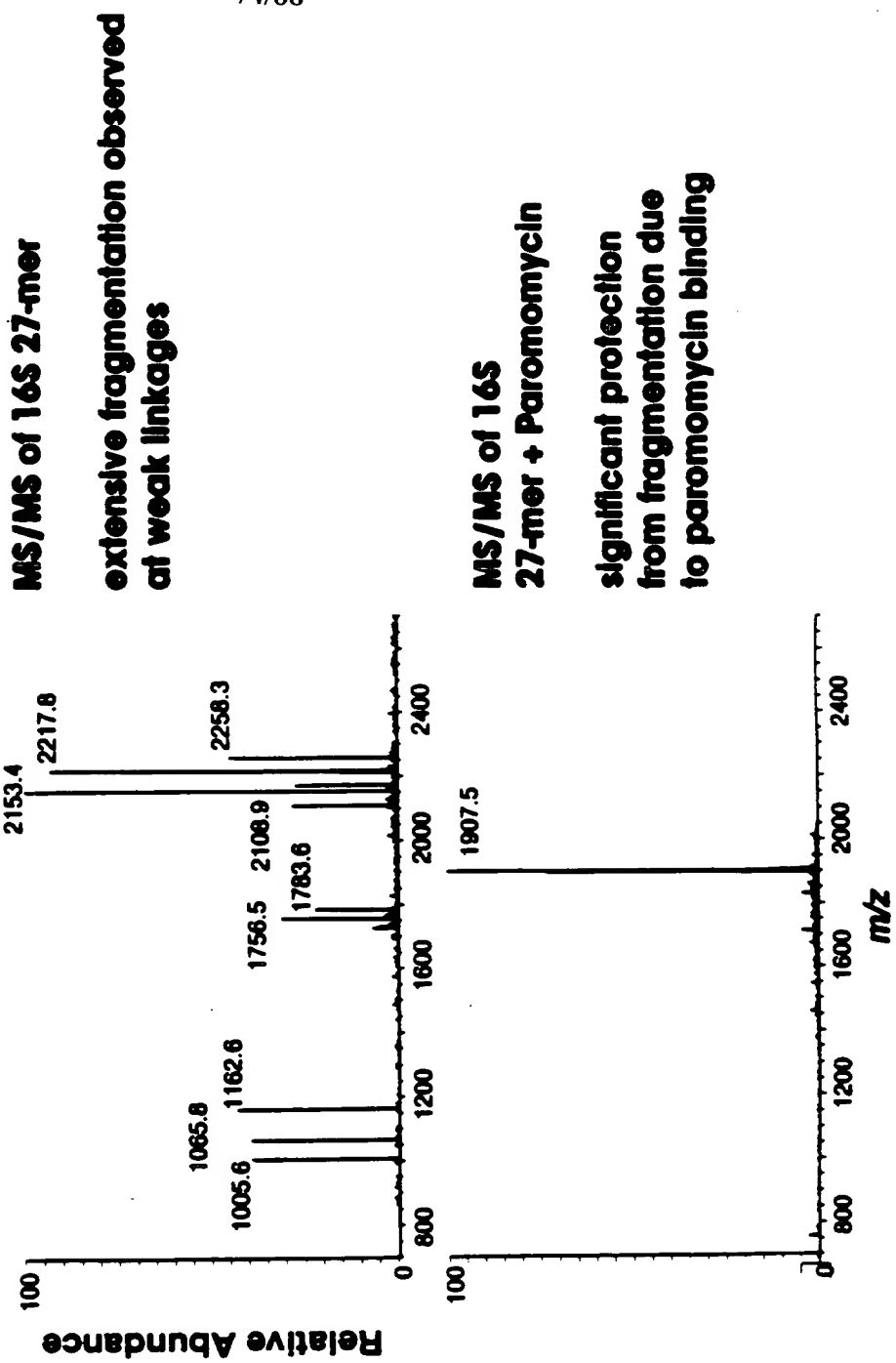


Figure 67

MASS Protection Assay

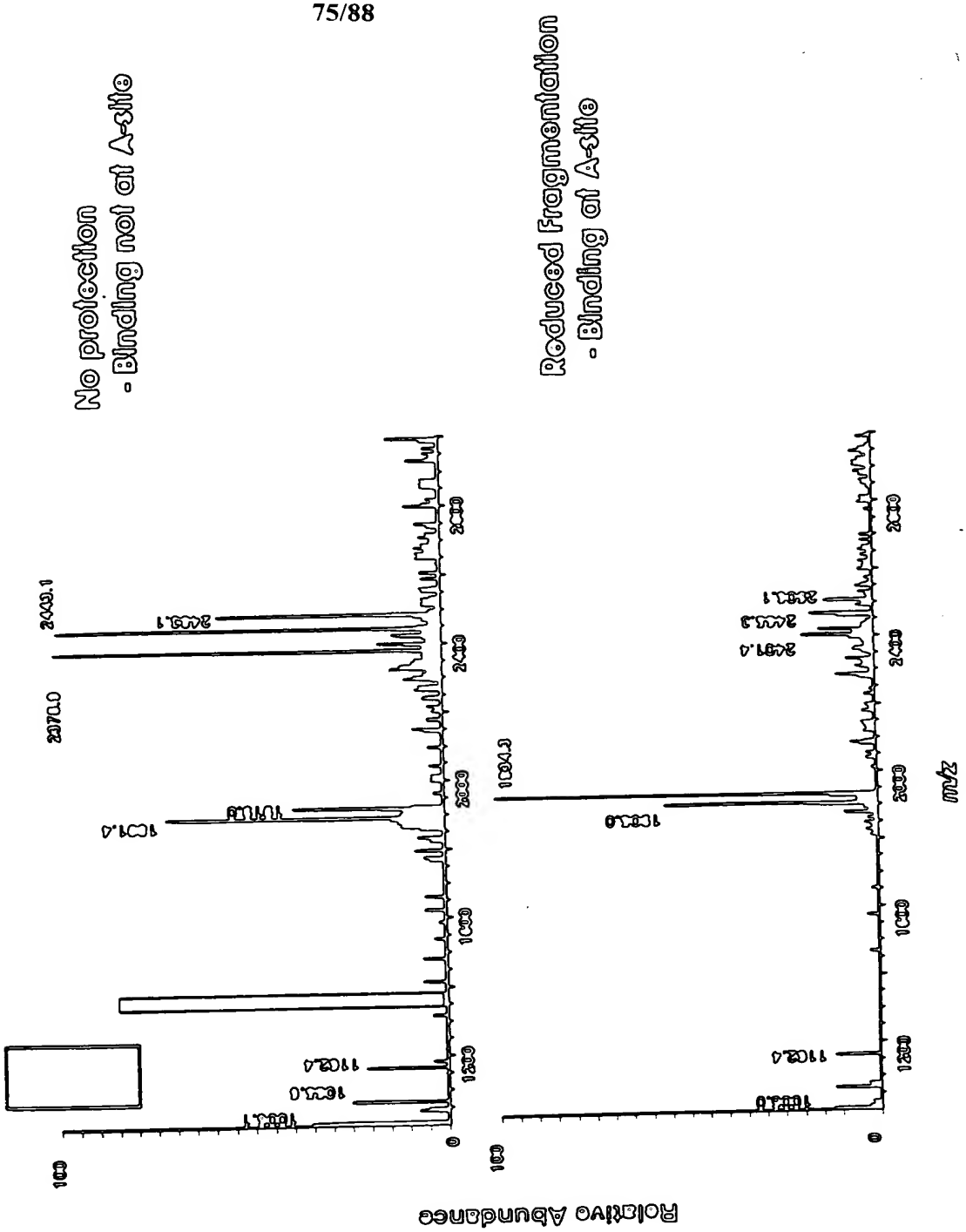


Figure 68

Eukaryotic and Prokaryotic A-Sites

Aminoglycosids antibiotics bind to A-site of decoding region in 16S RNA

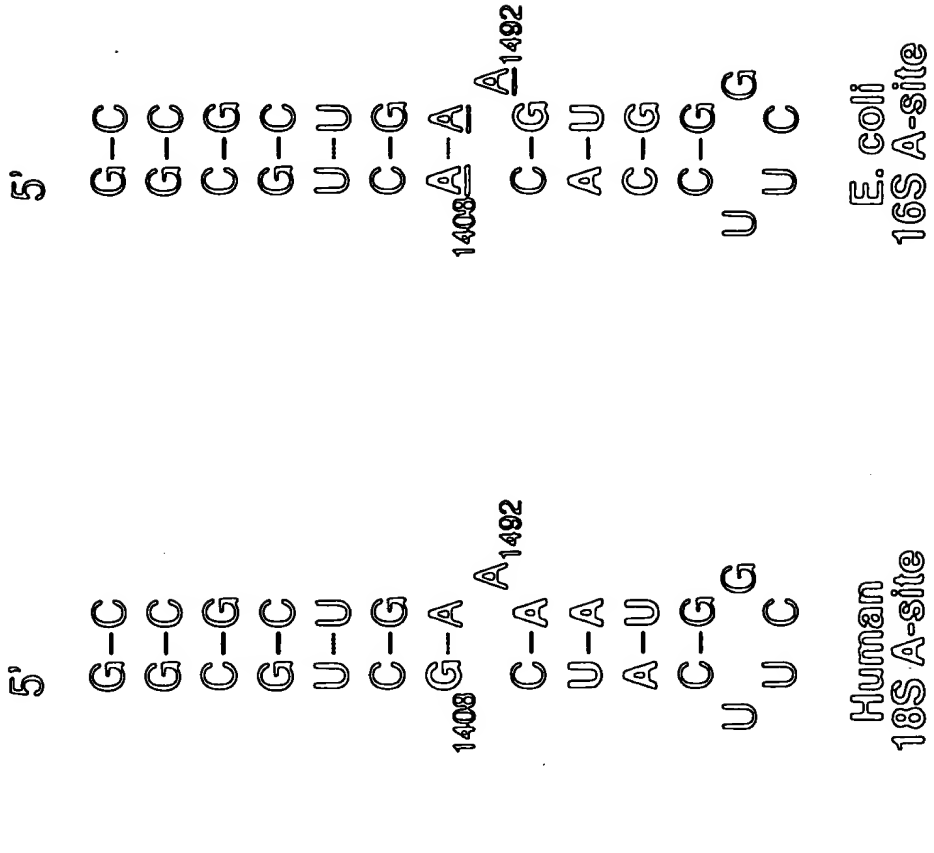


Figure 69
Neutral Mass Tag Does Not Affect Ligand Binding

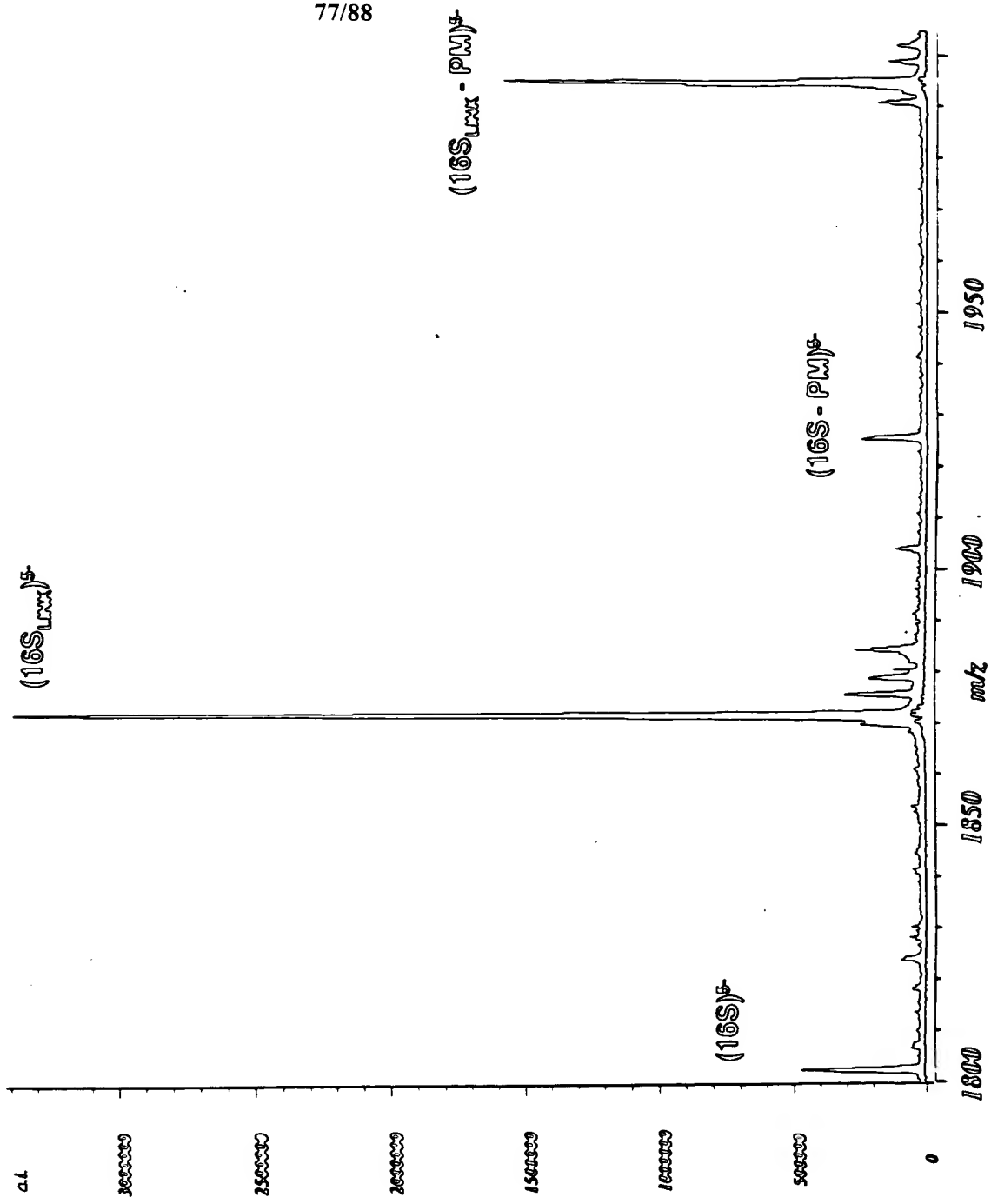
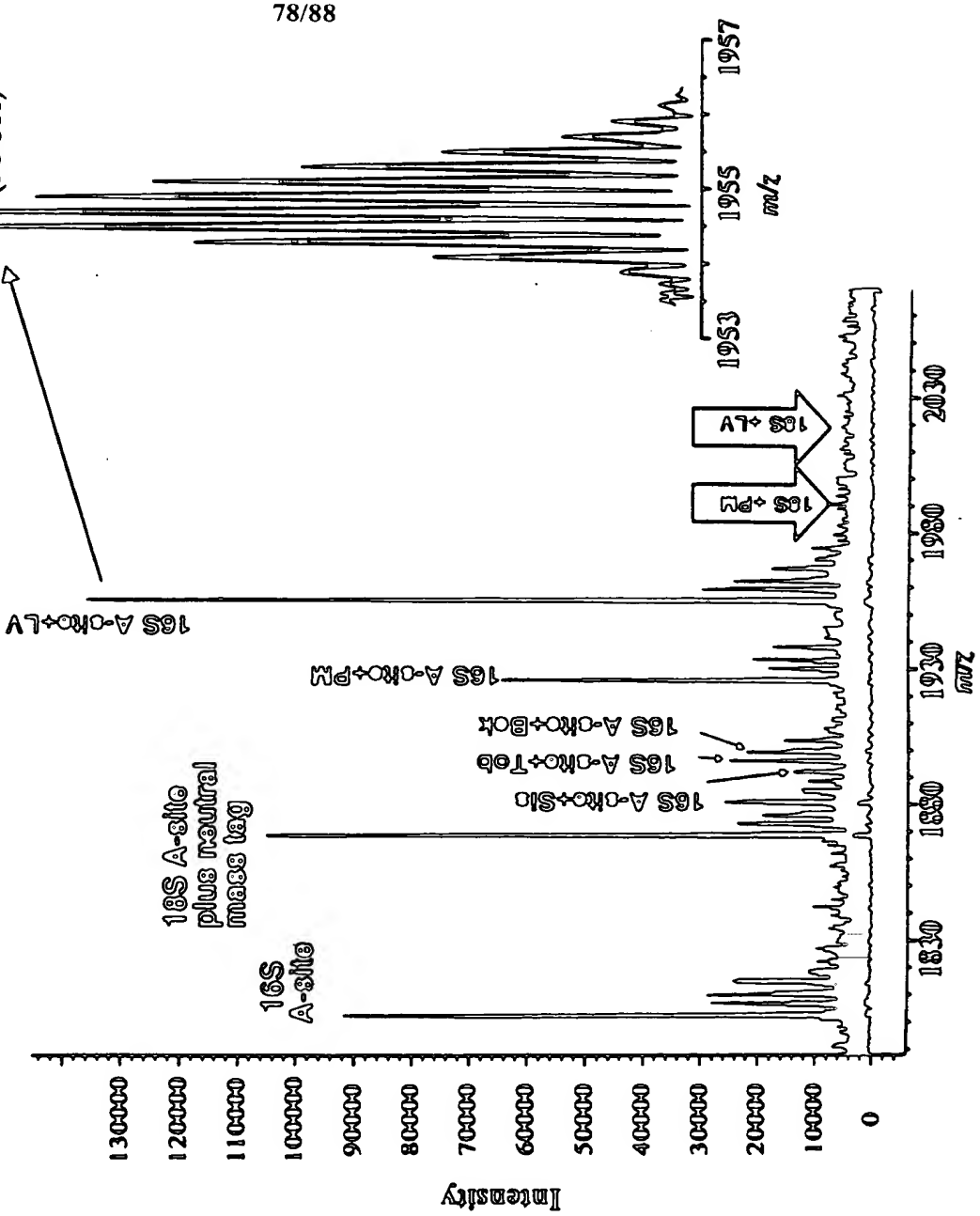


Figure 70

Simultaneous Screening of 16S A-site and 18S A-site Model RNAs Against Aminoglycoside Mixture



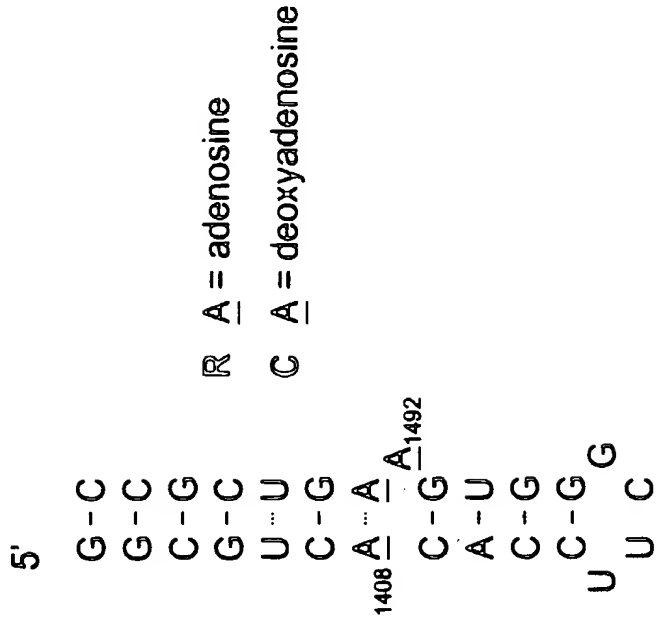
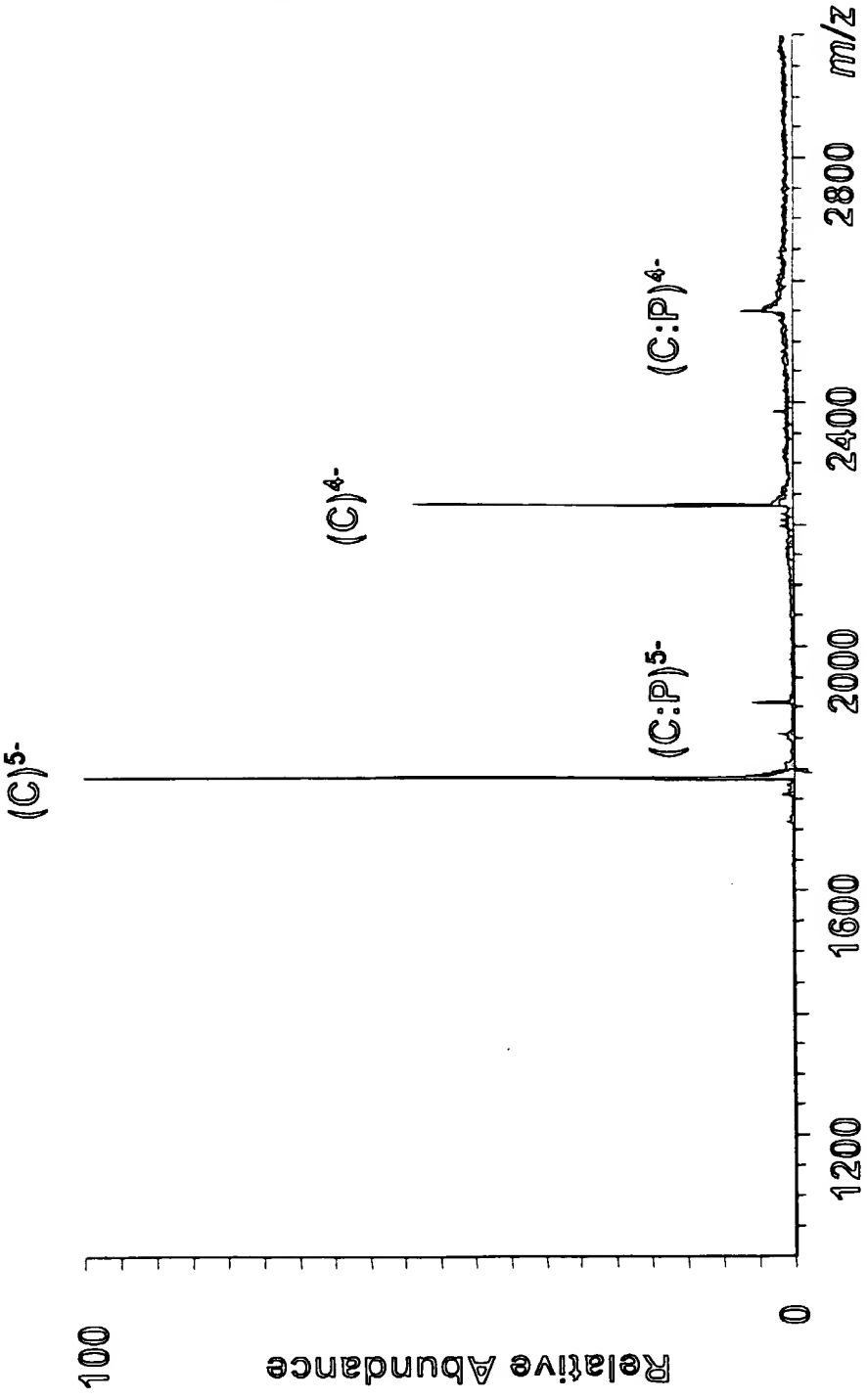

5'
G-C
G-C
C-G
G-C
U⋯U
C-G
A⋯A
1408 C-G
A-U
C-G
C-G
U C
A1492
G
R A = adenosine
C A = deoxyadenosine

Figure 71

Figure 72A


Relative Abundance
100
0
1200
1600
2000
2400
2800
m/z
(C)5-
(C)4-
(C:P)5-
(C:P)4-

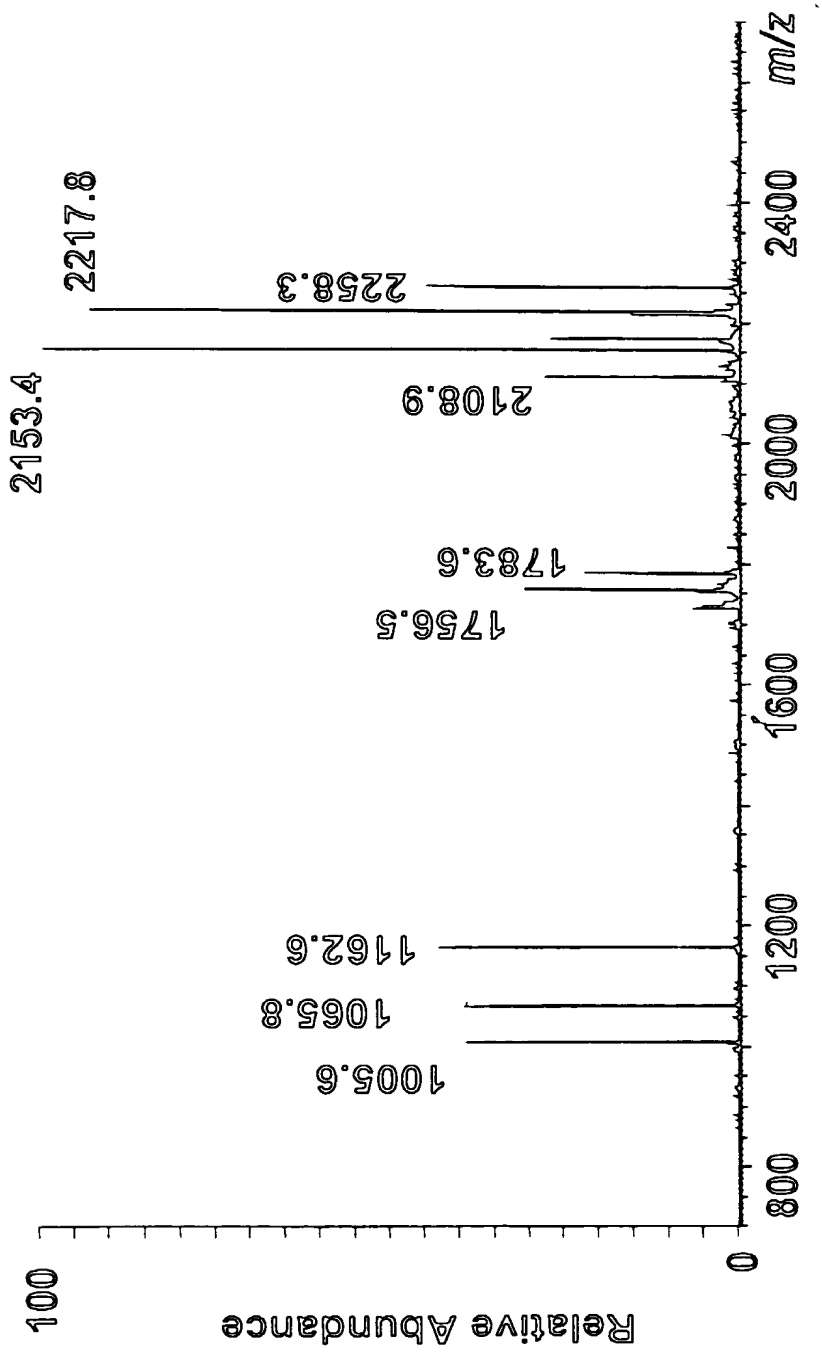

m/z
2400
2000
1600
1200
800
0
Relative Abundance
100
2217.8
2258.3
2153.4
2108.9
1783.6
1756.5
1162.6
1065.8
1005.6

Figure 72B

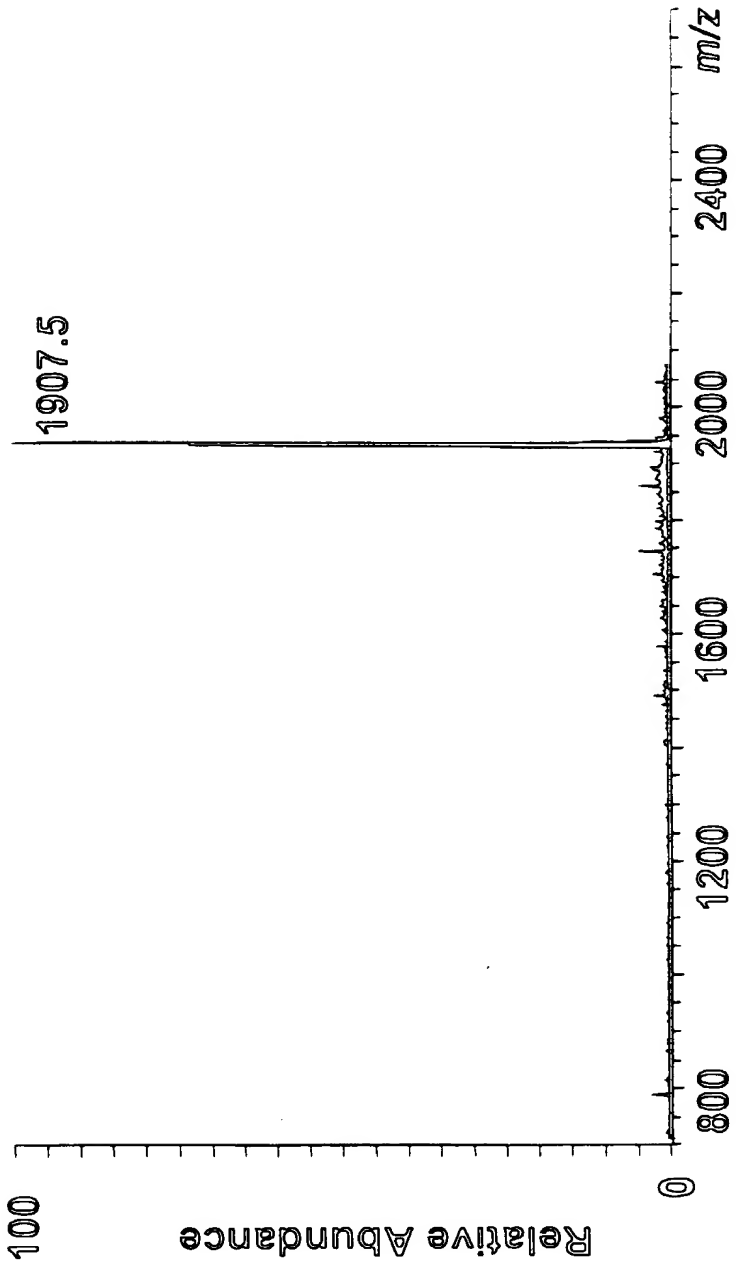


Figure 72C

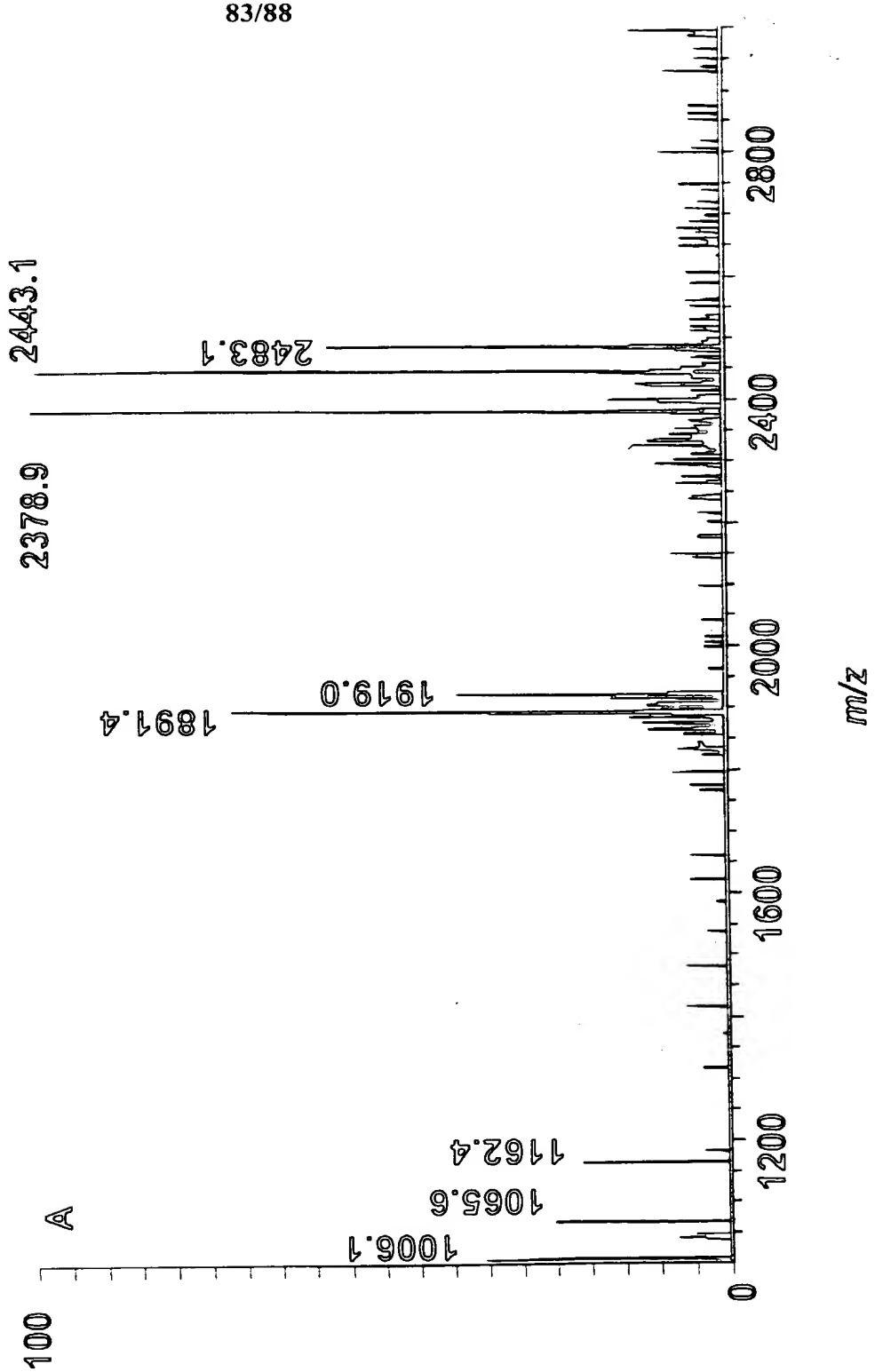

2443.1
2483.1
2378.9
1919.0
1891.4
1162.4
1065.6
1006.1
A
100
0
1200
1600
2000
2400
2800
m/z

Figure 73A

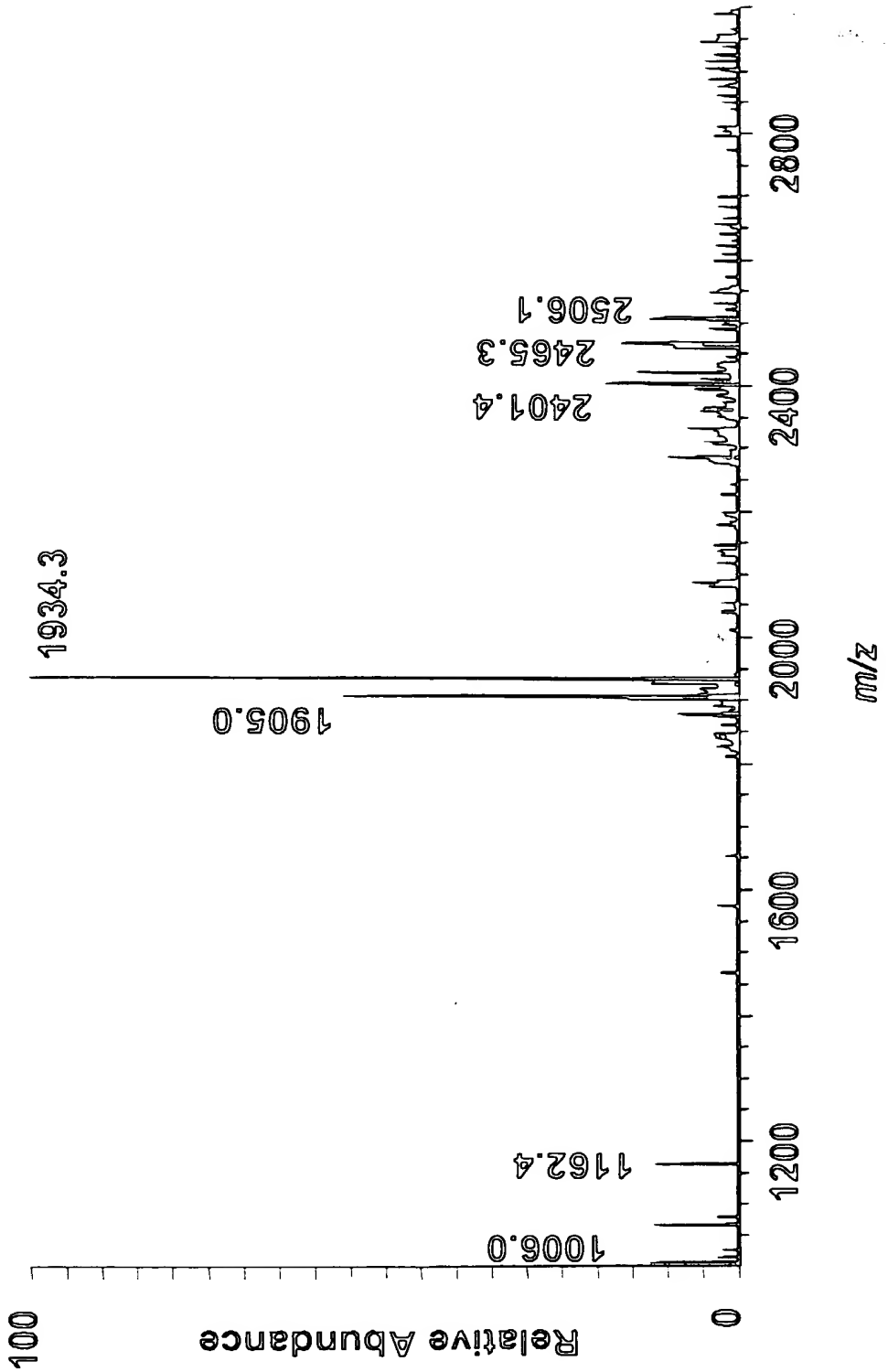

Relative Abundance
m/z
100
0
1006.0
1162.4
1905.0
1934.3
2401.4
2465.3
2506.1
1200
1600
2000
2400
2800

Figure 73B

85/88

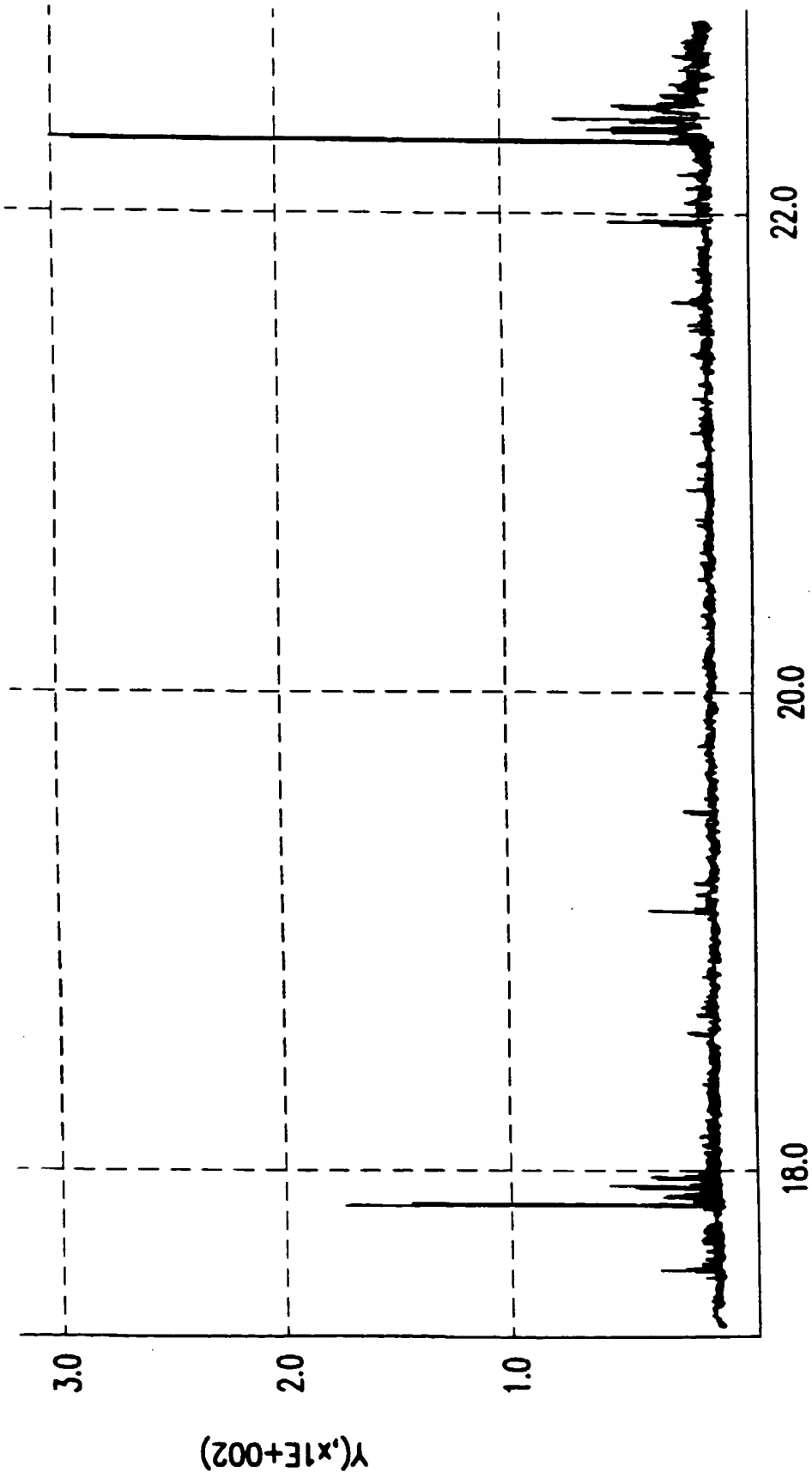


Figure 74

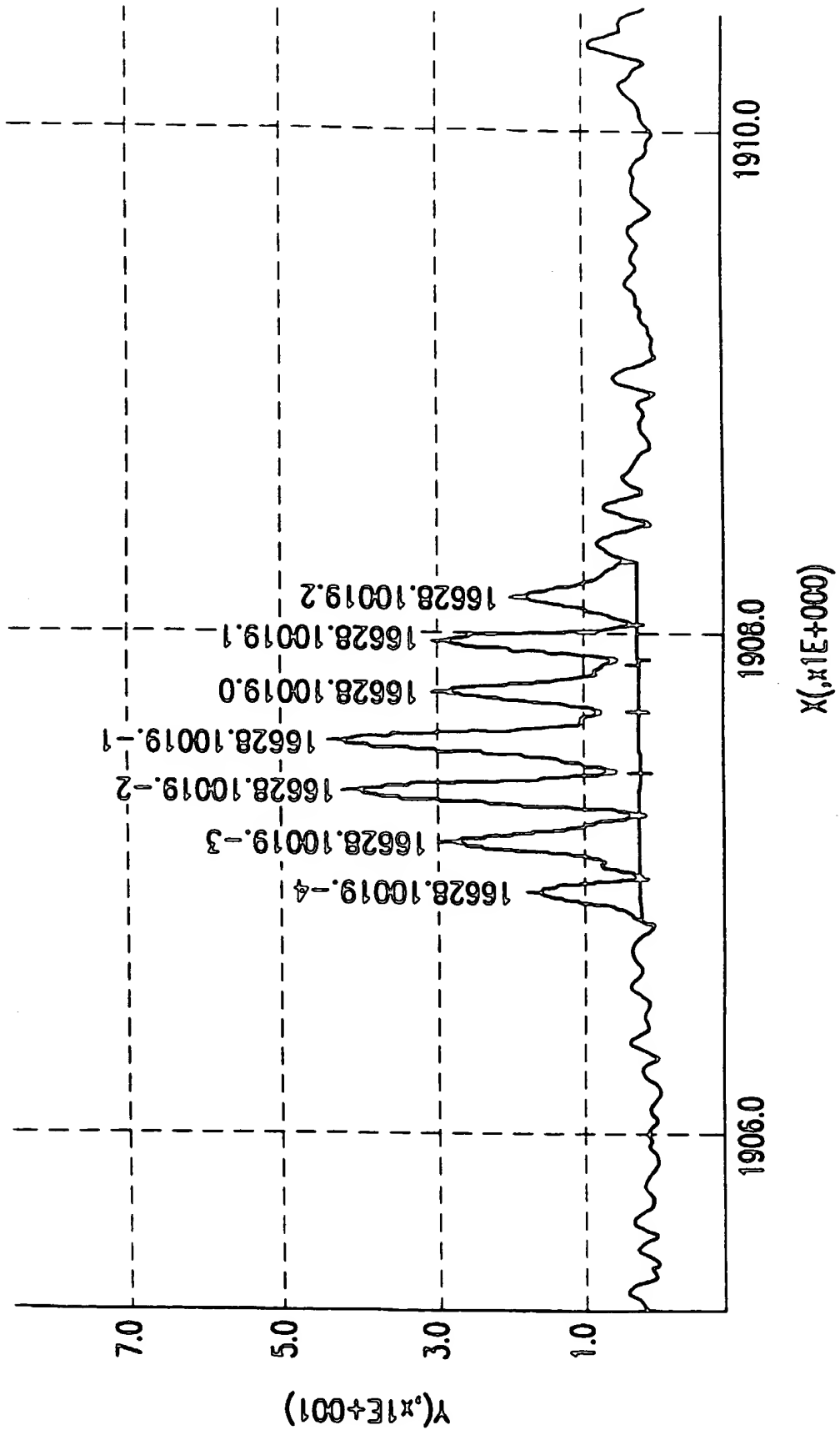

16628.10019.2
16628.10019.1
16628.10019.0
16628.10019.-1
16628.10019.-2
16628.10019.-3
16628.10019.-4
1910.0
1908.0
1906.0
X(,x1E+000)
7.0
5.0
3.0
1.0
Y(,x1E+001)

Figure 75

nr	name	apex	start	stop	height	area
1	16628-1.-4	1783.710	1783.635	1783.834	14.55	1.63
2	16628-1.-3	1783.909	1783.834	1783.972	60.04	5.15
3	16628-1.-2	1784.109	1784.021	1784.184	115.60	11.14
4	16628-1.-1	1784.308	1784.233	1784.383	167.34	15.89
5	16628-1.0	1784.508	1784.433	1784.620	133.94	14.74
6	16628-1.1	1784.707	1784.620	1784.795	136.60	13.38
7	16628-1.2	1784.907	1784.795	1784.982	82.63	8.56
8	16628-1.3	1785.107	1785.032	1785.219	57.81	5.21
9	16628-1.4	1785.306	1785.232	1785.369	32.31	2.65
10	16628-1.5	1785.506	1785.456	1785.569	17.67	1.12
11	16628.10019.-4	1906.974	1906.874	1907.031	12.63	1.00
12	16628.10019.-3	1907.173	1907.045	1907.273	22.54	2.11
13	16628.10019.-2	1907.373	1907.287	1907.444	33.86	2.91
14	16628.10019.-1	1907.572	1907.458	1907.701	34.87	3.30
15	16628.10019.0	1907.772	1907.701	1907.843	20.93	1.55
16	16628.10019.1	1907.972	1907.900	1908.043	21.03	1.55
17	16628.10019.2	1908.157	1908.086	1908.271	10.97	0.90
18	16628.-4	2229.874	2229.679	2230.029	27.51	4.87
19	16628.-3	2230.146	2230.029	2230.263	111.72	16.23
20	16628.-2	2230.380	2230.263	2230.516	225.18	32.39
21	16628.-1	2230.633	2230.516	2230.770	280.66	40.90
22	16628.0	2230.887	2230.770	2231.023	287.24	41.95
23	16628.1	2231.140	2231.023	2231.257	242.23	34.17

Figure 76

Docket No. IBIS-0171 Appln. No: 09/310,844
Title: MOLECULAR INTERACTION SITES OF INTERLEUKIN-2 RNA AND METHODS OF MODULATING THE SAME
Inventor(s): David J. Ecker, Richard Griffey, Stanley T. Crooke, Ranga Sampath, Eric Swayze, Venkatraman Mohan, Steven Hofstadler, John McNeil
Paul K. Legaard 215-568-3100
Sheet 87 of 88

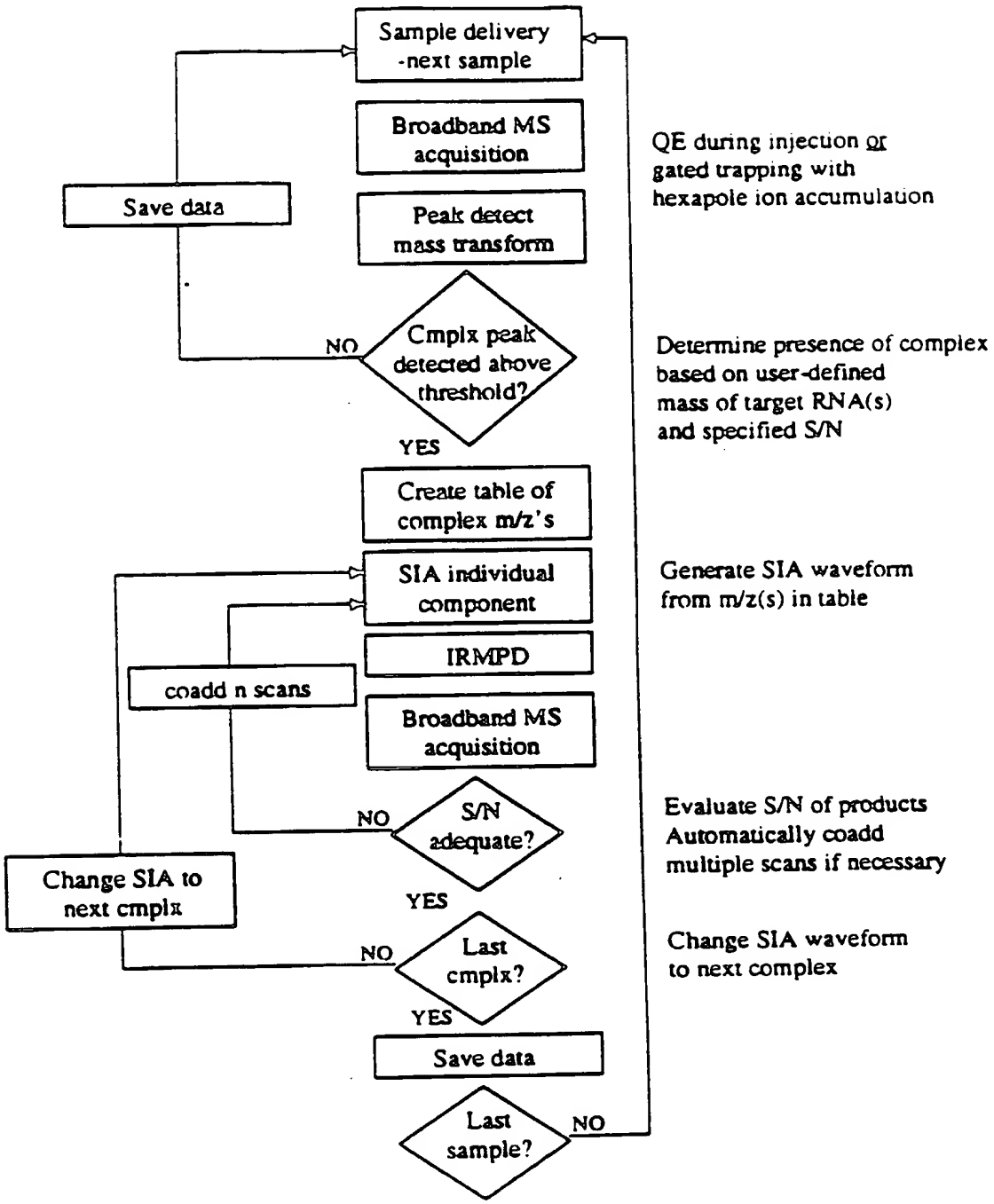

Sample delivery -next sample
Broadband MS acquisition
Save data
Peak detect mass transform
NO
Cmplx peak detected above threshold?
YES
Create table of complex m/z's
SIA individual component
IRMPD
coadd n scans
Broadband MS acquisition
NO
S/N adequate?
Change SIA to next cmplx
YES
NO
Last cmplx?
YES
Save data
Last sample?
NO
QE during injection or gated trapping with hexapole ion accumulation
Determine presence of complex based on user-defined mass of target RNA(s) and specified S/N
Generate SIA waveform from m/z(s) in table
Evaluate S/N of products Automatically coadd multiple scans if necessary
Change SIA waveform to next complex

Figure 77